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Abstract

Larvicidal activity of crude petroleum ether (PE), ethyl acetate (EtOAc) and methanol (MeOH) extracts of leaves and stem barks of *Sterculiaquinqueloba* (Garcke) K. Schum were testedagainst the late 3rd instar larvae of *Anopheles gambiae*, *Aedesaegypti* and *Culexquinquefasciatus*at 5 different concentrations50 – 800µg/ml. The larval mortality was observed after 24h, 48h and 72h of exposure. At 24h of exposure all extracts showed moderate larvicidal effects, except for methanolic extracts which showed very week effect at LC_{50}>750µg/ml.The highest larvicidal activityrecorded for leaf extracts against each species used were LC_{50}27.61µg/ml(EtOAc extracts), LC_{50} 43.9µg/ml(PE extracts) and LC_{50} 68.2µg/ml(EtOAc extracts) for *A. gambiae*, *C. quinquefasciatus* and *A. aegypti* respectively. Only EtOAc extract of stem against *A. gambiae* gave LC_{50}range between 50 - 100µg/ml. Generally leaf extracts showed higher activity than stem extract. This is an ideal eco-friendly approach aid for the control of mosquito species *A. gambiae, A. aegypti and C. quinquefasciatus.*

Key words: *Sterculiaquinqueloba*, larvicidal, Anopheles*gambiae*, *Aedesaegypti*, *Culexquinquefasciatus*

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Introduction

Several mosquito species which belong to genera *Anopheles*, *Culex*, and *Aedes* are principally the vectors of many of vector-borne diseases affecting human being and other animals in the world(Mohan and Ramaswamy, 2007). These diseases which cause millions of deaths to most of the tropical countries every year include, malaria, filariasis, Japanese encephalitis, dengue, chikungunya and yellow fever(Chowdhury et al., 2008, Kamaraj et al., 2009). According to Breman et al., (2007)and Scholte et al., (2003),*Anopheles gambiae*is one of the most efficient malaria vectors known around sub-saharan countries while *Culex quinquefasciatus*is known to transmit zoonotic diseases such as lympaticfilariasis, avian malaria and west Nile fever. On other hand *Aedesaegypti* is known to be primarily a vector of dengue, chingunya and yellow fever. According to Mweya et al., (2013)*A. aegypti* has been demonstrated in the laboratory to be capable of transmitting the virus causing Rift valley fever both mechanically and biologically.

Significant population of Tanzania community is at risk of being infected with these diseases some of which have major socio-economic implications. For instance, it is estimated that about 100,000 malaria deaths occur annually among all age group in Tanzania, 80% of which are deaths recorded from children less than 5 years(Mboera et al., 2008). Malaria is also implicated with decreased learning capacity to children and students in the 5 – 25 age range and in the loss of economic productivity in the workforce age range 15 – 55 years(Breman et al., 2007). Likewise patients (mostly children) with dengue fever and/or dengue haemorrhagic fever stay in hospital for approximately 5 – 10 days for severe cases and for mild cases dengue infection appears to be largely underdiagnosed in rural areas and patients are likely to be mistakenly treated with antimalarial drugs on the basis of clinical symptoms (Vairo et al., 2014). These diseases have created a number of health and economic problems among the Tanzanian community.

One of the approaches for control of these mosquito-borne diseases is the disruption of disease transmission through preventing the vector (mosquito) from biting the human

beings(Mohan and Ramaswamy, 2007). This approach can be either through using mosquito nets, repellents, killing the mosquito or causing larval mortality in a large scale at the breeding vector sites. The latter approach is attractive target for pesticides because mosquito breeds in water, and, thus, become easy to deal with them in this habitat (Chowdhury et al., 2008). Although the use of current conventional chemicals like malathion, DDT, organophosphate, organochlorine and pyre-throides has played a vital role in mosquito control, but they are never free from obstacles. In some parts of the world the use of these conventional chemicals has resulted in development of resistance(Patil et al., 2010, Mohan and Ramaswamy, 2007), affecting non targeted species some of which are beneficial insects (Park et al., 2012) and raised environmental and human health concerns(Scholte et al., 2003).

This problem has prompted the increased search for alternative insecticides all over the world. One of the best alternatives is the search for safe plant products with larvicidal activity because they are biodegradable in nature, easily available to local community and have no effect on non-targeted organisms that share the same habitat with mosquito larvae (Rawani et al., 2013). In this view of an increased interest of developing plant origin insecticides as an alternative to chemical insecticide, this study was undertaken to assess the larvicidal potential of the extracts of aerial parts of *S. quinqueloba*(Garcke) K. Schum tree species commonly known as large-leaved sterculia, against three mosquito larvae, *A. gambiae, A. aegypti* and *C. quinquefasciatus*. This plant is native to hot and dry regions of Sub-Saharan Africa with various medicinal values, including the use of its decoction of leaves and roots for the treatment of malaria (Louppe, 2008).

Material and Methods

Collection and Preparation of Plant Materials

The leaves and stem bark of *S. quinqueloba* were collected from Kigoma region around Gombe National Park and identified by Mr. Haji Seleman, a botanist from the Department of botany, University of Dar es Salaam and the voucher specimen (NMA 2141) was kept at Nelson Mandela African Institute of Science and Technology, Arusha. Samples were washed with water, dried in shade at room temperature and dried plant materials were then pulverized to get powders from which the extracts were prepared. 800g of powdered leaves and stem were sequentially extracted by maceration using petroleum ether (PE), ethyl acetate (EtOAc) and methanol (MEOH). After extraction the solvents were evaporated using vacuum evaporator to obtain the extract in dried form and then stored in airtight bottle and used for experiment.

Solvents, Reagents and Specimen

Methanol (absolute) was bought from FlukaChemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands) and Dimethyl Sulfoxide (DMSO) was purchased from RFCL Limited, Hayana, India. Petroleum ether and Ethyl acetate were purchased from (LobaChemiePvt Ltd, Mumbai, INDIA).The eggs of *A. gambiae, A. aegypti* and *C. quinquefasciatus*were collected from Tropical Pesticides Research Institute (TPRI), Arusha, Tanzania. The larvae were cultured and maintained in the laboratory at 27 $\pm$ 1^0C. Larval forms were maintained in tray by providing TetraMin tropical flakes.

Testing for larvicidal activity

The larvicidal test was performed according to World Health Organization (WHO) protocol with minor modification (WHO, 1996). Stock solutions (100 mg/mL) of each plant extract were prepared by dissolving them in DMSO. It was then diluted with distilled water to make 100 mL each, of 800, 500, 250, 100 and 50µg/mL solutions of each plant extract. Ten late third instar laboratory reared *A. gambiae, A. aegypti* and *C. quinquefasciatus* mosquito larvae were then introduced in the test solution and mortality was observed after 24 h, 48 h and 72 h. Negative control tests contained mosquito larvae, DMSO (0.5%) and water only. All tests were carried out in triplicate under controlled temperature (25 ± 2°C) and relative humidity of 75-85%. The larvae were considered dead if they were immobile and unable to reach the water surface when touched. Number of dead larvae was then recorded after 24 h, 48 h, and 72 h, and the mean percentage mortalities calculated for each concentration.

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} (lethal concentration) values and their 95% confidence limits were estimated by fitting a probit regression model observed on the relationship between percentage of mortality of larvae and logarithmic concentration of the substance. A student's t-test was performed to find the significance between some of the concentration of plant extract and mortality at different periods, and between some of the mortality observed in different plant extract with the control. All of the analysis was carried out using the SPSS (Statistical Package Social Science) software version 18.0.

Results and Discussion

Tables 1 – 3 show the larvicidal activity of leaf and stem extracts against late 3^{rd}instar larvae of *A. gambiae, A. aegypt* and *C. quinquefasciatus* after 24h, 48h and 72h exposure.

Student's t-test ofmethanolic extracts against the specimen tested did not show significant difference between treatment and control at 24h and 48h for the concentration used. The $LC_{50}$of methanolic extract at 24h and 48h provided values >750µg/mL which according to Komalamisra et al., (2005) they are regarded as non-toxic. All other data showed highly significant difference ($p<0.05$) between treatments and control, and the mortality observed was progressively increased with increasing extract concentrations.

Table 1: Larvicidal activity of leaf extracts against late 3rd instar larvae of *A.gambiae*after 24 h, 48h and 72h exposure

Specimen	Extracts	Time (h)	LC_{50}µg/ml (CI_{95})	LC_{90}µg/ml(CI_{95})	χ^2	Regression equation
A. gambiae	Ethyl acetate	24	178.9 (115.6 – 266.2)	526.3 (337.9 – 1295.6)	0.85	2.5x – 5.43
		42	29.81 (3.4 – 80.6)	199.3 (112.2 – 1410.9)	0.71	1.5x – 2.2
		72	27.61 (0.4 – 68.6)	129.8 (78.4 – 4059.1)	1.1	2.547x – 4.01
	Methanol	24	3762.4 (1932 – 7325)	63763 (32749 – 124147)		0.33x – 0.66
		48	788.5(558.8 – 1112.6)	3402.6(2411.5 – 4801.1)		0.62x – 1.32
		72	184.5 (121.1 – 273.6)	526 (339.9 – 1277.0)	1.23	2.815x – 6.25

As shown in the table 1 above, the leaf extract of EtOAc was more toxic than methanolic extract against *A. gambiae*and according to Komalamisra et al., (2005)EtOAc extract at 48h and 72h showed values that fall into very active range LC_{50}values <50µg/ml.

In table 2 below, EtOAc extract showed to be more toxic than PE extract against *A. aegypti*. But on the *C. quinquefasciatus*PE leaf extract showed slight high activity than EtOAc. Moreover, PE extract at 72h showed high activity against *C. quinquefasciatus*as it provided LC_{50} value <50µg/ml. The methanolic extract showed week activity for both *A. aegypt* and *C. quinquefasciatus*after 72h exposure, with LC_{50} value range from 200 - 750µg/ml.

Table 2: Larvicidal activity of leaf extracts against late 3rd instar larvae of *A. aegypti* and *C. quinquefasciatus* after 24 h, 48h and 72h exposure

Specimen	Extracts	Time(h)	LC_{50}µg/ml (CI_{95})	LC_{90}µg/ml(CI_{95})	χ^2	Regression equation
A. aegypt	Petroleum	24	290.8 (193.2 – 445.4)	861.4 (535.6 – 2572.2)	3.68	2.718x – 6.23
	ether	48	173.3 (98.4 – 280.8)	730.5 (410.9 – 2954.3)	1.79	2.051x - 4.10
		72	72.9 (38.7 – 108.6)	184.1 (120.8–734.7)	2.35	3.174x – 5.91
	Ethyl acetate	24	151.6 (104.9 – 214.9)	326.1 (227.4 – 703.9)	1.82	3.85x – 8.20
		48	84.5 (63.5 – 116.6)	127.0 (98.8 – 351)	0.03	7.25x –13.96
		72	68.2 (52.2 – 89.1)	99.9 (79.4 – 200.1)	0.01	7.7x – 14.12
	Methanol	24	3296.7(2078 – 5228)	23396(14751 – 37106)		0.47x – 0.12
		48	2890.1(1844 – 4528)	19388(30537 – 12436)		0.48x – 1.17
		72	644 (480.1 – 1135.8)	1241.2 (837.1 – 8545.9)	0.33	4.499x – 12.3
C. quinquefasciatus	Petroleum ether	24	105.6 (47.6 – 170.9)	460.8 (266.9 – 1728.1)	1.87	2.003x – 3.8
		48	58.0 (17.5 – 91.8)	185.8 (115.7 – 786.5)	0.95	2.535x - 4.47
		72	43.9 (0.5 – 69.4)	120.5 (76.7 – 6807.6)	0.47	2.925x – 4.80
	Ethyl acetate	24	139.8 (41.1 – 273.9)	1264 (518.5–48439.9)	1.25	1.34x – 2.74
		48	55.1 (2.2 – 116.9)	525.5 (243.2 – 16593.9)	1.13	1.3x – 2.21
		72	53.3 (2.5 – 112.2)	474.9 (225.6 – 10368.4)	1.22	1.35x – 2.3
	Methanol	24	8716(3789 – 20048)	299738(130321– 689399)		0.26x – 0.53
		48	964.3(646.3 – 1478)	5271.3(3533 – 7864.8)		0.54x – 1.11
		72	439.4 (301.7 – 686.7)	1133.8 (715.2 – 4103.8)	0.70	3.113x – 8.02

Stem extracts of *S. quinqueloba*were also used to evaluate the activity against the mentioned specimen of mosquitoes. PE extract was not used for larvicidal activity due to solubility problem. As shown in the table 3 below, methanolic extracts of stem showed

lower activity compared to EtOAc extracts. The response of all mosquitoes species against methanolic extracts were not effective at 24h and 48h of exposure time with LC_{50} values >750 µg/mL but showed week activity after 72h exposure with LC_{50} values range from200-750µg/mL.

Table 3: Larvicidal activity of stem extracts against late 3rd instar larvae of *A. aegypti*,*C. quinquefasciatus* and *A. gambiae* after 24 h, 48h and 72h exposure

Specimen	Extracts	Time (h)	LC_{50}µg/ml (CI_{95})	LC_{90}µg/ml(CI_{95})	χ^2	Regression equation
A. aegypti	Ethyl acetate	24	227.3 (149.9 – 335.4)	642.0 (415.9 – 1607.1)	2.25	2.842x – 6.58
		48	168.7 (115.5 – 240.7)	390.2 (267.6 – 853.4)	3.62	3.518x – 7.73
		72	143.3 (93.9 – 209.1)	374.1 (247.9 – 889.0)	3.23	3.075x – 6.43
	Methanol	24	3304.8(806 – 1286)	23453 (14788 – 37197)		0.47x – 1.15
		48	1018.8(806.7 – 1286)	2749.8(2177 – 3472)		0.92x – 2.29
		72	680.2(555.9– 864.9)	925.5 (768.0 – 2030.4)	0.98	9.586x – 26.85
C. quinquefasciatus	Ethyl acetate	48	263.4 (180.8 – 375.8)	632.9 (431.1 – 1414.3)	1.96	3.367x – 8.14
		72	210.2 (139.7 – 307.5)	571.5 (375.2 – 1365.7)	2.79	2.951x -6.75
	Methanol	24	2125(1220 – 3699)	22407(12870 – 39010)		0.39x – 0.801
		48	1269.3(788.4 – 2043)	9583.4(5952 – 15429)		0.47x – 0.914
		72	219.2 (43.1 – 1095.9)	4480.8 (974.7 – 19000)	1.23	0.978x – 2.03
A. gambiae	Ethyl acetate	24	135.4 (90.9 – 193.8)	313.7 (214.6 – 711.3)	3.50	3.511x – 7.48
		42	90.7 (45.2 – 140.7)	263.7 (192.7 – 625.6)	1.42	2.379x – 4.66
		72	60.6 (22.3 – 91.9)	167.3 (107.9 – 537.4)	0.11	2.906x – 5.17
	Methanol	24	1362.7(826 – 2247.1)	11404(6915 – 18805)		0.85x – 0.43
		48	792.7(539.9 – 1163)	4061.7(2767 – 5962.5)		0.56x – 1.13
		72	207.8 (126.1 – 329.3)	782.3 (456.9 – 2648.2)	0.16	2.226x – 5.06

Much research has been conducted on plant derived chemicals which are non-toxic to man and domestic animals and serve as useful basis for the development of safer and

more selective insecticides for mosquito control (Komalamisra et al., 2005). Among the most preferred target for mosquito control is larva stage, because larva has low mobility in nature and hence making treatment to be easily localized in time and space as compared to adult stage(Kumar and Maneemegalai, 2008). Moreover, the fight against adult mosquito is a temporary phenomenon, somehow very unsatisfactory especially to large area and it contributes much into environmental pollution (Chowdhury et al., 2008). Therefore, since secondary compounds of plants make up a vast repository of compounds with a wide range of biological activities, plant origin insecticide with larvicidal potential might be one of the best alternatives for controlling mosquito species.

This desire of searching and subsequently using the natural and ecofriendly compounds for larvicidal activity is also expected to contribute into sustainable development to most of the developing countries where death of millions of people are recorded every year due to mosquito-borne diseases (Kumar et al., 2014). The use of plant products for vector control can be used with minimum care by local communities at lowest cost possible due to readily availability of plants in specific situations and hence reducing the cost and risk to human harmful products originated from synthetic pesticides(Kamaraj et al., 2009). Due to the mentioned larvicidal potential of plant products, this paper screened the potential of leaf and stem extracts of *S. quinqueloba* against *A. aegypti, A.gambiae* and *C.quinquefaciatus.*

This paper showed that, leaf extracts of PE and EtOAc gave high potency with low critical lethal concentrations (LC_{50}<50 µg/mL) against late 3rd instar larvae of *A. gambiae* and *C.quinquefasciatus*(Table 1 and 2). Stem extract showed lower activity compared to leaf extract and most of the responses recorded range from 200 – 100 µg/mL which is termed as median potency activity according to Kumar et al., (2014). Methanolic extracts of both leaf and stem gave week potency against all mosquito species tested, the LC_{50}observed range from 200 – 750µg/mL. Moreover the leaf extracts of EtOAc exhibited high activity compared to stem extract of EtOAc against the mosquito larvae tested. The LC_{50} for 72h exposure time of leaf and stem extract of EtOAc against *A. gambiae, A. aegypti* and *C. quinquefasciatus* were 27.61, 68.2, 53.3 and 60.6, 143.32, 210.22µg/ml respectively. These varying results of lethal concentration observed, indicated that plant parts and solvent used for extraction, have an effect on the differences of levels of toxicity among the insecticidal ingredients of this plant.

The most reported insecticidal group of compounds from plants with larvicidal activity against various species of mosquito are steroids, flavonoids, phenols, tannins, terpenoids, carbohydrates and saponin (Elimam et al., 2009, Gopieshkhanna and Kannabiran, 2007). Chapagain et al., (2008) reported that saponin extracted from the fruit

of *Balanitesaegyptica* showed 100% mortality against larvae of *A. aegypti.* According to Hostettmann and Marston (2005), saponins are freely soluble in both organic solvents and water, and work by interacting with the cuticle membrane, which is the most probable reason for larval death. Aluminium chloride obtained from alder leaf, known for its phenolic complexing activity, is also reported to have the larvicidal activity against *A. aegypti*(David et al., 2001). The impact of phenolic compounds on mosquito larvae particularly *A.aegypti* and *A.gambiae*has been reported by Marston et al., (1993).

Isoflavonoids from tubers of *Neorautaneniamitis* had a larvicidal effect against the malaria and filariasis transmitting mosquitoes, *A. gambiae* and *C. quinquefaciatus* respectively (Joseph et al., 2004). The role of tanning extracted from *Hemidesmusindicus*, *Gymnemasylvestre*and *Eclipta prostrate* which causes mortality in *C. quinquefasciatus* larvae has been also discussed by Khanna and Kannabiran (2007). Alkaloid compounds from aqueous extracts of *Ricinus communis* leaves and *Tetraclinis articulate* wood showed strong toxic activity against *C. pipens, A. caspius* and *Anopheles* species (Taha et al., 2011).

Sterculia species have been found to have various classes of compounds such as alkaloids, flavonoids, tannins and saponins (Wang et al., 2003). Thus, presence of these compounds may potentiate that the shown larvicidal activity of *S. quinqueloba*leaf and stem extracts may be due to the presence of the mentioned classes of compounds.

Conclusion

Based on the above results *Sterculiaquinqueloba* (Garcke) K. Schum has shown paramount larvicidal importance. The use of plants for larvae control offers a safer alternative compared to synthetic chemical. The extracts or isolated bioactive compounds from the plant could be used in stagnant water bodies which are known to be breeding sites for mosquitoes. However, further studies on the identification of the active compounds, their mode of action and field trials are recommended.

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