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Mosquito larvicidal activity of polar extracts from three *Kotschya* species against *Anopheles gambiae s.s*

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Abstract
Anopheles gambiae is a main vector for transmission of malaria parasites. In endemic places, malaria contributes significantly to high mortalities and poverty. Synthetic insecticides command the widest application in malaria vector control but are non-biodegradable and currently facing resistance. In searching for alternative insecticides, this study evaluated the larvicidal potential of polar extracts from leaves and roots extract of *Kotschya thymodora, Kotschya strigosa and Kotschya speciosa* against *Anopheles gambiae*. The bioassay was done following WHO protocols (2005) in which 20 healthy third instars larvae were exposed to plant extracts of concentrations ranging from 12.5µg/mL to 200µg/mL for 48hours. *Kotschya thymodora* extracts exhibited the highest activity (LC_{50}, leaves 16.35µg/mL, and roots 53.35µg/mL) followed by *Kotschya strigosa* (LC_{50}, leaves 37.08µg/mL, and roots 237.31µg/mL) and *Kotschya speciosa* (LC_{50}, leaves 75.85µg/mL, and roots 252.03µg/mL). The findings indicates that *Kotschya* species can serve as potential alternative source for safe and ecofriendly insecticides for malaria control.

Keywords: Larvicidal activity, *Anopheles gambiae, Kotschya thymodora, Kotschya strigosa,* Kotschya speciosa

1. Introduction
Mosquitoes play the major role in the transmission of some protozoal and viral diseases such as malaria, lymphatic filariasis, Japanese encephalitis, Yellow fever, Zika fever and Dengue fever. These diseases are continuing to be a major health problem in the developing countries [1] Amongst these mosquito borne diseases malaria presents the highest cause of morbidity and mortalities to people living in endemic areas [2] The World malaria report of 2019 shows that in 2018 about 405,000 people died of malaria worldwide, 70% of which being children under 5 years of age [3]. In endemic countries, malaria contributes to a substantial economic loss in capital incomes. The costs incurred by the households for prevention, diagnosis and treatment is the major cause of poverty in these places [4]. For sustainable socio-economic development of people who are living in developing countries that are located in malaria endemic regions, elimination of malaria is therefore important. The vector control methods that target mosquito eggs, larva and adult *Anopheles gambiae* accounts for the commonly used technique. Synthetic and semi-synthetic insecticides have found a wide application for this purpose owning to its high use in insecticide treated nets (ITNs) and indoor residual spraying (IRS). The efficiency of those insecticides is slowly decreasing due to recently reported increase of mosquito resistance towards the commonly used insecticides such as pyrethroid which is the only insecticide used in ITNs [3, 5]. Moreover, some synthetic insecticides are non-biodegradable hence leading to environmental pollution in line with disturbance to the ecosystem [6]. Nevertheless, vector control is still ranked as the most efficient method for malaria control since the attack of immature stages in breeding sites offers more significant target [7]. It is therefore necessary to develop efficient and eco-friendly larvicides for this purpose. Plants are rich source of a wide number of chemical entities (phytochemicals) that can be studied to provide better alternatives to the existing insecticides [8]. The crude extracts of some plants belonging to the *Fabacae* family are reported to possess larvicidal activities against various mosquito species. *Kotschya uguenensis* is reported to exhibit larvicidal activity against *Anopheles gambiae* larva [9] while *Kotschya thymodora, Kotschya strigosa* and *Kotschya*

speciosa are reported to exhibit activity against the larva of *Culex quinquefasciatus* [10] This study investigated further the larvicidal effect of *Kotschya thymodora*, *Kotschya strigosa* and *Kotschya speciosa* against the third instar larva of *Anopheles gambiae*.

2. Materials and Methods

2.1 Plant Material collection: The plant materials were collected from the central and southern highland regions of Tanzania in October 2013. The materials were authenticated by Haji Seleman, a botanist from Department of Botany at the University of Dar es salaam. *Kotschya thymodora* (Voucher specimen number FMM3628) was collected from Njombe region at GPS coordinates 9056' 11.2 E 34034 '52.6. *Kotschya strigosa*,(Voucher specimen number FMM 3629) and *Kotschya speciosa*, (Voucher specimen number FMM 3626) were collected from Iringa region at GPS coordinates 8030' 39.9 E 35010' 10.8; and 8030' 39.9 E 35010' 10.8 respectively [11]. The materials were dried under shade then ground into course powder and macerated in 80% ethanol/water for 72 hours with occasional shaking. The crude mixture was then filtered and concentrated using rotary evaporator at 30 ºC then stored in the refrigerator.

2.2 Mosquito Larva: The test larvae were obtained from the insectary of Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Sciences. The strain originated from a colony reared by National Institute for Medical Research (NIMR), Amani Research Centre in Tanga region of Tanzania. 1st instar larva were fed on yeast while 2nd and 3rd instar larva were fed on fish food (tertamin). Larva were reared in distilled water and the room temperature was mentioned between 28 ± 2 ºC.

2.3 Larvicidal activity: The larvicidal activity was done following the WHO protocol of 2005 [12] with some minor modifications. The stock solution (10,000 µg/mL) was prepared by dissolving 1 g of the dried plant extract in 1mL of DMSO then making it to 100 mL by adding distilled water. From this, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL and 200 µg/mL solutions were prepared in 250mL beakers for testing. For all the assays 20 healthy third instars larvae were placed in 100 mL of the appropriate concentration in distilled water. Four replicates of each concentration and equal number of controls were set up simultaneously. Dimethyl sulfoxide (DMSO) at 1% v/v in distilled water was used as a negative control experiment. All tests were carried out three times using different sample solutions and different batches of mosquito larvae at the same stage. The tests were conducted at a room temperature maintained at 28 ± 2 ºC. The cumulative mortality data were recorded after 24 and 48 hours after exposure.

2.4 Statistical analysis: The arithmetic means of the cumulative percentage mortalities with the estimates of standard errors were calculated using Microsoft excel (2013). Probit analysis was used to estimate the LC_{50} and LC_{90} from the regression analysis done by using Graphpad prism (version 5.0). The obtained mortalities were subjected to further statistical analysis using student's t-test, analysis of variance (ANOVA) and Tukey multiple comparison test.

3. Results

Results revealed potential larvicidal activity of both leaf and root extracts of the three plants against *Anopheles gambiae* larva (Table 1). Amongst the leave extracts, the highest percentage mortality was recorded from *Kotschya thymodora* followed by *Kotschya strigosa* and lastly *Kotschya speciosa* (Figure 1 and 2). Statistical analysis however shows no significant difference ($P \leq 0.05$) between the mean mortalities observed from the leaves of *Kotschya thymodora*, and *Kotschya strigosa* at different levels of concentrations and for both 24 and 48 hours exposure time. The roots extracts demonstrated comparatively lower mortalities relative to leaves for the same plant. *Kotschya thymodora* demonstrated the highest activity among the root extracts followed by roots of *Kotschya strigosa* whose activity was not statistically significant different from that of *Kotschya speciosa* ($P \leq 0.05$) at all levels of test concentrations. However, at the lowest test concentration (12.5 µg/mL), the mortalities observed for *Kotschya strigosa* roots and these from *Kotschya speciosa* roots were not statistically different for both 24 and 48 hours exposure time (Figure 3 and 4). Furthermore, statistical analysis at $P \leq 0.05$ shows mortalities recorded from the individual plant extract increased significantly from 24 to 48 hours exposure time. The negative control experiment showed no mortalities recorded for both 24 and 48 hours of exposure indicating that dimethyl sulfoxide (DMSO) had no direct toxic effect onto the larval death but the contents of the extracts.

Table 1: LC_{50} and LC_{90} of the leaves and root extracts from three *Kotschya* species recorded after 48 hours post exposure.

Plant extract	LC_{50} (µg/mL) (LCL – UCL)	LC_{90} (µg/mL) (LCL – UCL)	Regression equation	R^2
K. thymodora leaves	16.35 (1.41 - 31.29)	55.99 (5.84 - 106.14)	Y = 2.151x + 2.443	0.84
K. thymodora roots	53.35 (8.99 - 97.71)	242.82 (59.44 - 545.04)	Y = 2.016x + 1.538	0.98
K. strigosa leaves	37.08 (21.09 - 53.09)	104.66 (47.42 - 162.02)	Y =2.728x + 0.696	0.78
K. strigosa roots	237.31 (163 - 311)	1814.93 (989 - 2641)	Y = 1.496x + 1.564	0.88
K. speciosa eaves	75.85 (61.9 - 89.7)	583.02 (119.14 - 1285.34)	Y = 1.552x + 2.088	0.97
K. speciosa roots	252.03 (172.03 -332.03)	2221.43 (1401.43 - 3041.43)	Y = 1.311x + 1.84	0.90

LC_{50} and LC_{90} - Indicates lethal concentrations that kills 50 and 90% of exposed larva, respectively, LCL – UCL are Lower and Upper class limits at 95% confidence level respectively. Number of replicates is three.

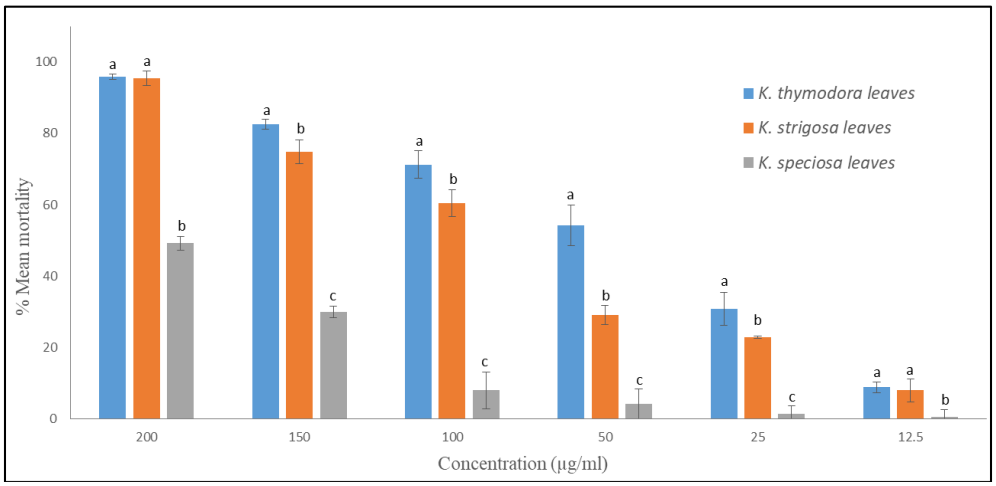

Fig 1: Bioactivity of leave extracts after 24 hours exposure time. Values for percentage mortalities are mean ± standard error. Columns with the same letter at a certain level of concentration are not significantly different at $P \leq 0.05$, unpaired student t test.

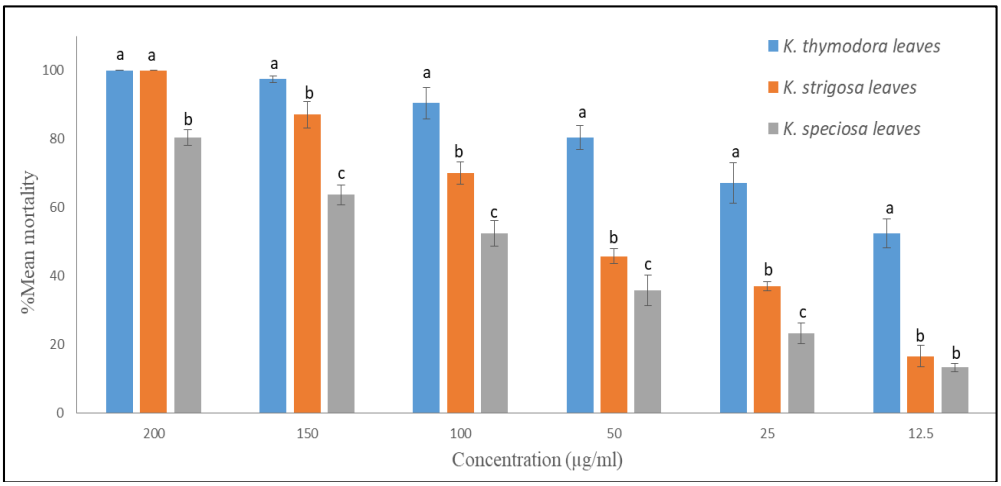

Fig 2: Bioactivity of leave extracts after 48 hours exposure time. Values for percentage mortalities are mean ± standard error. Columns with the same letter at a certain level of concentration are not significantly different at $P \leq 0.05$, unpaired student t test.

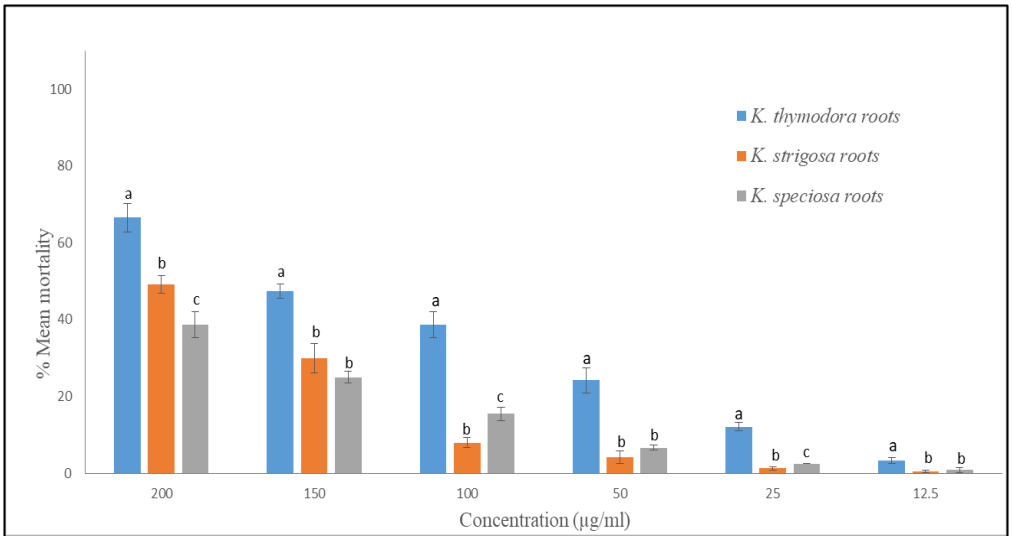

Fig 3: Bioactivity of root extracts after 24 hours exposure time. Values for percentage mortalities are mean ± standard error. Columns with the same letter at a certain level of concentration are not significantly different at $P \leq 0.05$, unpaired student t test.

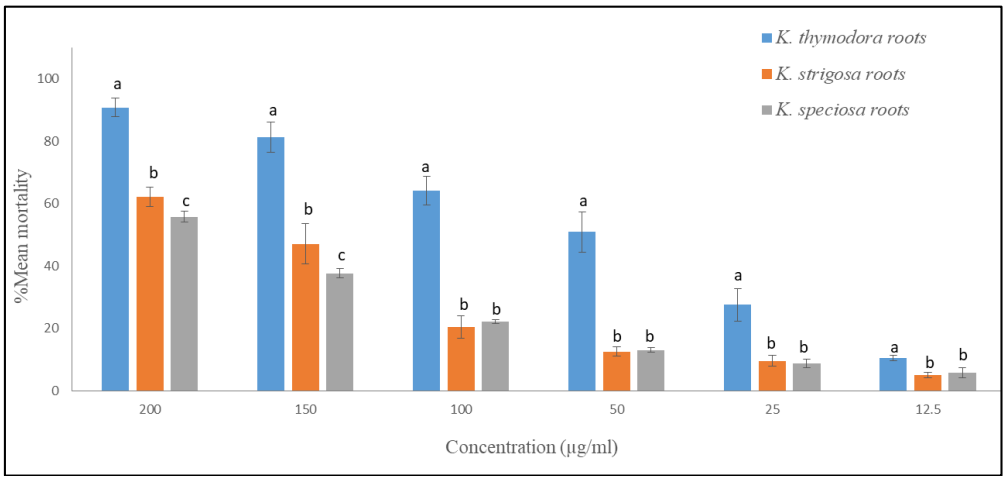

Fig 4: Bioactivity of root extracts after 48 hours exposure time. Values for percentage mortalities are mean ± standard error. Columns with the same letter at a certain level of concentration are not significantly different at $P \leq 0.05$, unpaired student t test.

4. Discussion

The present study report on the larvicidal activity of the leaves and roots from the three *Kotschya* species against the third instar *Anopheles gambiae* larva. The results displays a remarkable difference in the bioactivity, in relation to the concentration of the plant material used, part of the plant used and time of exposure. The overall mortalities of the six plant extracts under the study suggest higher larvicidal potentials from the leaves compared to roots (Table 1). This can be caused by the presence of different levels or types of secondary metabolites (bioactive compounds) between these two parts of the plant [13]. Biosynthesis of secondary metabolites is known to be under the influence of both biotic and abiotic factors such as soil contents and environmental conditions. Therefore, leaves and roots of the same plant may contain different phytochemical profile leading to difference in the level of the active component [14]. The increased mortalities after a prolonged exposure of 48hours may be due to several factors including molecular size, physical and chemical properties of the active constituents of the crude extract, that may influence rate of absorption and permeation through the membranes and hence its bioavailability [15].

Several other studies have reported on the larvicidal potentials of the plants from the genus *Kotschya.* The roots and stem barks of *Kotschya uguenensis* are known to cause protrusion of the gastro-intestinal contents through the anal pores of *Anopheles gambiae* larva that eventually results in death upon prolonged exposure [9]. *Kotschya thymodora, Kotschya strigosa and Kotschya speciosa* are reported to be active against the larva of *Culex quinquefasciatus.* These studies indicated that 500 µg/mL of the roots and stem barks is necessary for 70% mortalities after 8 days exposure time [16]. The present study however, shows much lower concentration (150 µg/mL) is needed to cause higher mortality effect in *Anopheles gambiae.*

Isolation of compounds from the three plant species have resulted to a number of bioactive compounds [11, 17]. A recent study has reported cycloartenone to be the major triterpenoid ketone isolated from the ethanolic extract of both *Kotschya thymodora, Kotschya strigosa and Kotschya speciosa.* The compound however was also reported to be mild active against *Anopheles gambiae* larva [11]. *Kotschya strigosa* polar extracts revealed the presence of a high content of phenolic and flavonoid compounds along with the isolation of a potent antioxidant isoflavonol kotstrigoisoflavanol, *β*-sitosterol, *β*-sitosterol *3-O-β-D*-glucopyranoside and diosmetin [17]. *β* -sitosterol is known to possess a potent larvicidal activity against *Aedes aegypti, Culex quinquefasciatus* and *Anopheles stephensi* [18] *β*-sitosterol *3-O- β -D*-glucopyranoside is known to be a potent insecticide against *Aedes aegypti* [19] The insecticidal activity of diosmetin is not well documented in the literature but it is known to be a part of the phytochemical contents of some insecticidal plants such as *Mentha piperita* and *Blumea balsamifera* [20, 21] Apart from cycloartenone literature review reveals no further information about isolation of compound from *Kotschya thymodora* and *Kotschya speciosa.* Nevertheless, the aerial parts of *Kotschya speciosa* is reported to be used traditionally for its antifungal and antiprotozoal effects against *Plasmodium falciparum* [22]

5. Conclusion

The findings of this study show that leave and root extracts of the three *Kotschya* species, *Kotschya thymodora, Kotschya strigosa and Kotschya speciosa* can serve as potential alternatives source of insecticides that can be further studied to provide safe and eco-friendly compounds in the control of mosquito borne diseases such as malaria. The active compound (s) contributing to the larvicidal activity of these plant extracts is still not known. This research work is opening a way for further research on isolation and characterization of the chemical constituents in these plant species.

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7. References

1. National Malaria Control Programme, WHO, Ifakara Health Institute and the INFORM Project (2013). An epidemiological profile of malaria and its control in mainland Tanzania. Report funded by Roll Back Malaria and Department for International Development-UK, 2013
2. Ayele D, Shifa M. Socio-economic Determinants of

Poverty in Malaria Endemic Areas of Kenya. Current Journal of Applied Science and Technology. 2017; 22(4):1-14.
3. World malaria report 2019. Geneva: World Health Organization, 2019. Licence: CC BY-NC-SA 3.0 IGO.
4. Teklehaimanot A, Mejia P. Malaria and poverty. Annals of the New York Academy of Sciences. 2008; 1136:32-7.
5. Yewhalaw D, Wassie F, Steurbaut W, Spanoghe P, van Bortel W, Denis L *et al*. Multiple Insecticide Resistance: An Impediment to Insecticide-Based Malaria Vector Control Program. PLoS One. 2011; 6(1).
6. Hakeem KR, Akhtar MS, Abdullah SNA. Plant, soil and microbes. Springer International Publishing, Switzerland, 2016; 1:1-366.
7. Kamareddine L. The biological control of the malaria vector. Toxins. 2012; 4(9):748-67.
8. Rahman S, Barman NC, Ferdous T, Biswas SK. Plant Extract as Selective Pesticide for Integrated Pest Management. Biotechnological Research. 2016; 2(1):6-10.
9. Innocent E, Joseph CC, Gikonyo NK, Nkunya MHH, Hassanali A. Growth disruption activity of polar extracts from *Kotschya uguenensis* (Fabaceae) against *Anopheles gambiae* s.s. (Diptera: Culicidae) larvae. International Journal of Tropical Insect Science. 2008; 28(4):220–4.
10. Innocent E, Kaale E, Mbwambo Z. Larvicidal activities of five *Kotschya* species against *Culex quinquefasciatus* Say (Culicidae: Diptera). International Journal of Biological and Chemical Sciences. 2012; 6(2).
11. Innocent E, Sempombe J, Masimba PJ, Daniel I, Heydenreich M. Comparison of cycloartenone from four insecticidal *Kotschya* species (Fabaceae) harvested during dry and wet seasons. Pharmacognosy and Phytochemistry. 2015; 4(4):97-102.
12. WHO Guidelines for Laboratory and Field Testing of Mosquito Larvicides. WHO/CDS/WHOPES/GCDPP, 13, 10-12, 2005.
13. Pavarini DP, Pavarini SP, Niehues M, Lopes NP. Exogenous influences on plant secondary metabolite levels. Animal Feed Science and Technology. 2012; 176(1-4):5-16.
14. Nath S, Rawat S, Rawal RS, Bhatt ID, Pathak B, Fulekar MH. Soil constituents influence accumulation of phytochemicals and nutritional content in *Wrightia tinctoria* of North Gujarat, India. Indian Journal of Plant Physiology. 2017; 22(2):197-205.
15. Shinoda W. Permeability across lipid membranes. Biochimica et Biophysica Act. 2016; 1858(10):2254-65.
16. Innocent E, Kaale E, Mbwambo Z. Larvicidal activities of five *Kotschya* species against *Culex quinquefasciatus* Say (Culicidae: Diptera). International Journal of Biological and Chemical Sciences. 2012; 6(2):603-12.
17. Awouafack MD, Tchuenguem RT, Ito T, Dzoyem JP, Tane P, Morita H. A New Isoflavanol from the Fruits of *Kotschya strigosa* (Fabaceae). Helvetica Chimica Acta. 2016; 99(4):321-4.
18. Ghosh A. Efficacy of phytosterol as mosquito larvicide. Asian Pacific Journal of Tropical Disease. 2013; 3(3):252.
19. Kashte S, Walke S, Parwe N, Mulani R. Agnimantha: An herbal larvicide and pupicide against malarial vector *Anopheles stephensi*. International Journal of Mosquito Research. 2015; 2(3):89-93.
20. Biniaś B, Gospodarek J, Rusin M. Effect of water extract from mint on selected crop pests feeding and survival. Journal of Ecological Engineering. 2017; 18(2):119-28.
21. Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X *et al*. *Blumea balsamifera*- A phytochemical and pharmacological review. Molecules. 2014; 19(7):9453-77.
22. Zofou D, Tene M, Ngemenya MN, Tane P, Titanji VPK. In Vitro Antiplasmodial Activity and Cytotoxicity of Extracts of Selected Medicinal Plants Used by Traditional Healers of Western Cameroon. Malaria Research and Treatment. 2011, 1-6.