

Proceedings

Bioactivity of Essential Oils of *Laggera pterodonta* and *Laggera aurita* against Larvae of *Anopheles gambiae*, Malaria Vector [†]

Fatima Dantanko ^{1, *} and Yoila D. Malann ²

¹ National Biotechnology Development Agency, Abuja, Nigeria

² Biological Sciences Department, University of Abuja

* Correspondence: fatimadantanko@yahoo.com

† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: <https://iecps2020.sciforum.net/>.

Published: 30 November 2020

Abstract: The role of plants as sources of biologically active entities cannot be over emphasized. Plants belonging to different families having mosquitocidal activity have been discovered from an established ethnobotanical and laboratory based studies. Biological control of mosquitoes using botanicals remains the safest and environmentally sound alternative to chemical control. The essential oil of two Nigerian *Laggera* species, *L. pterodonta* and *L. aurita* were investigated against the fourth instar larvae of the malaria vector, *Anopheles gambiae*. WHO protocol was adopted for the larvicidal bioassay. Three replicates comprising of 20 larvae each were exposed to various concentrations of the essential oil. Larval mortality was observed after 24 and 48 h respectively. The results showed that mortality increased with increase in concentration and period of exposure. The essential oil of *L. pterodonta* was found most effective with LC₅₀ values of 418 and 404 mg/L after 24 and 48 h respectively while the essential oil of *L. aurita* recorded LC₅₀ value of 688 and 642 mg/L after 24 and 48 h respectively. The GC-MS results revealed the essential oil of *L. pterodonta* comprises 50.83% of compounds that have been reported to have larvicidal activity while the essential oil of *L. aurita* comprises of 43.69% compounds with larvicidal activity. The better activity of *L. pterodonta* essential oil could be attributed to it having higher percentage of compounds like γ -Terpinene and 4-Carvomenthenol with larvicidal activity. The results suggest that the essential oil of the plants have the potential to be used as eco-friendly approach for the control of mosquitoes.

Keywords: *Laggera pterodonta*; *L. aurita*; Essential oil; Larvicides; *Anopheles gambiae*

1. Introduction

The importance of medicinal plants lies not only in their chemotherapeutic value in traditional health care but also in their potential as sources of biologically active entities (Maharaj et al., 2011). In Africa in general and Nigeria in particular, indigenes have used certain plants or parts of plants for thousands of years as insecticides. Plant essential oils, crude extracts or their chromatographic fractions have shown to have various levels of bioactivity against different developmental stages (larval, pupal and adult) of mosquitoes (Mudalungu, 2013). Larviciding is more effective than control of adult due to limited area of larvae movement compared to free flying adult (Ohimain et al., 2014). However, intensive use of synthetic insecticides in mosquito control programmes has created widespread resistance (Ombito et al., 2014), undesirable effects on other insects and negative impacts on the environment (Ohimain et al., 2014). These problems have highlighted the need for exploration and use of plant products with insecticidal properties for mosquito control.

Plant essential oils may be an alternative source of mosquito larvicides since they constitute a rich source of bioactive compounds that have different mode of action and are biodegradable. In fact many researchers have reported on the effectiveness of plant essential oils against mosquito larvae and the recent examples are studied by Ombito et al. (2014), Ohimain et al. (2014), Sergio et al. (2018).

The genus *Laggera* belongs to the family asteraceae and consists of about 20 species. Plant in the genus *Laggera* are aromatic and mostly found growing as weed in Africa and Asia. Previous researches have shown efficacy of the plants in the genus *Laggera* against various insects; this includes insecticidal activity against maize weevil (Langsi et al., 2017), mosquito larvicidal activity (Ollengo et al., 2016) as well as mosquito repellent and oviposition deterrent (Singh and Mittal, 2015). *Laggera pterodonta* and *Laggera aurita* are the two common species found growing in Nigeria. They are used mainly as antispasmodics, diuretic, laxative and antidysentric (Singh and Mittal, 2015).

This study aimed at determining the larvicidal activity of the essential oil of *Laggera pterodonta* and *Laggera aurita* against the Nigerian medically important malaria vector mosquito, *Anopheles gambiae*.

2. Materials and Methods

2.1. Plant Collection

The plants (*Laggera aurita* and *L. pterodonta*) were collected from Chaza village, Suleja Local Government of Niger State, Nigeria. The plants were identified, authenticated and assigned voucher specimen number of NIPRD/H/6977 and NIPRD/H/6978 for *Laggera aurita* and *Laggera pterodonta* respectively by a botanist at the Medicinal Plant Research and Traditional Medicine (MPR & TM) Department of National Institute of Pharmaceutical Research and Development (NIPRD).

2.2. Essential Oil Extraction

The essential oils were extracted at MPR and TM Department (NIPRD). Each fresh plant (*Laggera pterodonta* and *Laggera aurita*) were sorted out and cut into small pieces that were meant to increase the surface area during the heating process. A two (2) litre round bottomed flask was packed with the plant pieces (including water) connected to Clevenger apparatus and placed on a heating mantle. Hydro-distillation process was carried out for about six (6) hours according to the British pharmacopoeia (Papachristos and Stamopoulos, 2004). This process was repeated several times in order to reach the required amount. The essential oils obtained were dried over anhydrous sodium sulphate to ascertain the yield as *w/w*. The oil was then stored in sealed glass vials at 4 °C until chemical composition analysis and bioassay (Omoriegbe et al., 2012).

2.3. Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil were analysed by GC-MS using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy = 70 eV), scan range of 45–700 amu and scan rate of 3.99 scans/s] and Shimadzu GCMS solution data system at National Institute for Pharmaceutical Research and Development, Abuja Nigeria. The gas chromatography column was HP-5ms fused silica capillary with 5% phenyl-methylpolysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for gas chromatography oven temperature was 60–160 °C at a rate of 15 °C/min, then held at 160 °C for 2 min, followed by 160–280 °C at a rate of 10 °C/min and then finally held at 280 °C for 2 min. The injector port temperature was 250 °C while detector temperature was 280 °C. Diluted sample (1/100 in hexane, *v/v*) of 1.0 µL was injected using autosampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with NIST Mass Spectral Library (NIST 08). The percentages of each component are reported as raw percentages based on the total ion current without standardization (Okhale et al., 2018).

2.4. Collection and Rearing of Mosquitoes

Mosquito larvae were collected from plastic containers containing water that was placed outside. It was taken to the Biology Laboratory of Department of Biological Sciences, University of Abuja and identified by using keys provided by Gilles and DeMeilon (1968). It is important to note that *Anopheles* larvae were identified by the absence of siphon and they stay parallel to surface of water. Larvae identified were transferred into a beaker. The beaker was covered with net (polynestrene). The larvae were maintained by feeding them with larval food (ground fish). Within 2–3 days the larvae were transformed into pupae and then adult emerged and were found hanging on the net. The adult mosquitoes were identified up to species level by consulting keys produced by Gilles and De Meilon (1968).

2.5. Bioassay of Essential Oils of *L. pterodonta* and *L. aurita*

The essential oils were tested against early 4th instar larvae. The essential oils were weighed and solubilized in dimethylsulphoxide kept at 1%. The bioassay was conducted according to WHO (2015b) protocol. Twenty (20) healthy 4th instar larvae were introduced into a beaker containing 20 mL water and test essential oil. The experiment was carried out in triplicate and Control was run simultaneously. Mortality was recorded after 24 h and 48 h while percentage mortality was calculated from an average of the three (3) trials.

2.6. Statistical Analysis

SPSS 16.0 version package was used for analysing the data. Probit analysis was used to determine the LC₅₀ and LC₉₀ at 95% confidence limits of upper confidence limit and lower confidence limit.

3. Results

3.1. Chemical Composition of the Essential Oils by GC-MS

The GC-MS analysis of the essential oils of *Laggetera pterodonta* and *Laggetera aurita* are shown in Table 1. A total of 44 chemical components were the identified and quantified from the essential oil of *Laggetera aurita*. The major components were Benzene, 2-tert-butyl-1,4-dimethoxy (25.24%), Caryophyllene (12.25%) and γ -Terpinene (8.92%). On the other hand, a total of 45 constituents were identified from the essential oil of *Laggetera pterodonta*. The major components were γ -Terpinene (12.72%), Benzene,2-tert-butyl-1,4-dimethoxy (12.66%), (+)-4-Carene (10.40%), 4-Carvomenthenol (9.22%), and γ -Eudesmol (8.67%). Upon comparing the chemical composition of essential oil of the two plants (*L. pterodonta* and *L. aurita*), it was observed that both plants had these eighteen compounds in common; α -Phellandrene, (+)-4-Carene, o-Cymene, γ -Terpinene, β -Linalool, 2-Carene, Linalool, 4-Carvomenthenol, p-Menthan-8-ol, Thymol methyl ether, Benzene, 2-tert-butyl-1,4-dimethoxy, Caryophyllene, α -Caryophyllene, 1,2-Benzenediol, O-(4-butylbenzoyl), Cadina-1(10),4-diene, α -Bourbonene, tau-Muurolol and Phthalic acid, cyclobutyl tridecyl est.

Table 1. Chemical composition of *Laggetera pterodonta* and *Laggetera aurita* essential oil by GC-MS.

ID	Compound	Percentage (%)	
		<i>L. pterodonta</i>	<i>L. aurita</i>
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5	0.33	-
2	Tricyclo[4.1.1.0(2,5)] octane	-	0.15
3	trans-beta-Ocimene	1.48	-
4	Nortricyclyl bromide	-	0.16
5	Benzenepropanoyl bromide	0.93	-
6	Sabinene	0.47	-
7	s-Triazaborane	-	0.04
8	α -Phellandrene	2.36	4.36

9	(+)-4-Carene	10.40	5.58
10	o-Cymene	1.85	0.64
11	β -Methylmercaptoethylamine	-	0.10
12	α -Limonene	0.93	-
13	Hyacinthin	0.68	-
14	γ -Terpinene	12.72	8.92
15	β -Linalool	2.25	0.43
16	2-Carene	3.46	1.38
17	Linalool	2.51	1.16
18	1-(2-methylphenyl)-Ethanone	0.26	-
19	4-Isopropyl-1-methyl-2-cyclohexane	0.80	-
20	3(10)-Carene-4-ol, acetic acid esters	0.53	-
21	6-Methylenebicyclo[3.1.0]hexane	0.18	-
22	1,4-Dimethyl- δ -3-tetrahydroace	0.39	-
23	Pyrrole-2-aldehyde	0.14	-
24	4-Carvomenthenol	9.22	3.43
25	p-Menthan-8-ol	0.31	0.07
26	γ -Acetopropanol	0.06	-
27	2,3,4,5-Tetrahydropyridazine	0.06	-
28	Dimethylethylbenzene	0.34	-
29	Thymol methyl ether	0.62	2.19
30	4-Ethylformanilide	-	0.06
31	Trifluoromethyl peroxy nitrate	-	0.03
32	2,2'-azobis[2-methyl propionitrile	-	0.27
33	1-methylpyrrole	-	0.04
34	Benzene, 2-tert-butyl-1,4-dimethoxy	12.66	25.24
35	Caryophyllene	5.83	12.25
36	α -Caryophyllene	3.29	2.69
37	Isolongifolan-8-ol	2.34	-
38	1,2-Benzenediol, O-(4-butylbenzoyl)	0.05	0.20
39	(2S,4R)-p-Mentha-[1(7),8]-diene 2-1	-	0.60
40	Cadina-1(10),4-diene	0.84	-
41	α -Bourbonene	0.78	-
42	Caryophyllene oxide	1.16	-
43	Ethane, 1-(2-bromoethoxy)-2-methoxy	0.18	-
44	Cyclohexane, butylidene	0.20	-
45	γ -Eudesmol	8.67	-
46	2-Isopropenyl-1,3-dimethylcyclopentane	-	1.34
47	δ -Cadinol,(-)-	2.03	-
48	α -Cadinol	-	5.29
49	γ -Eudesmol	1.42	-
50	α -Muurolene	-	0.30
51	tau-Muurolole	1.94	5.91
52	Cedrol	-	1.51
53	Cadina-1(10),4-diene	0.84	4.20
54	(+/-)-Camphor	-	1.29
55	Champaca camphor	1.95	-
56	Juniper camphor	2.40	-
57	Benzenepropanoyl bromide	-	0.03
58	2,4-Diisopropenyl-1-methylcyclohexane	-	1.30
59	Borazine	-	0.04
60	α -Bourbonene	0.78	6.30

61	1,11-Dodecadiyne	-	0.34
62	3,5-Dithiono-6-methyl-1,2,4-triazine	-	0.08
63	Isopiperitenone	-	0.06
64	Patchoulane	-	0.83
65	s-Triazine	-	0.03
66	(-)-Spathulenol	-	0.92
67	3-Buten-1-one, 2,2-dimethyl-1-phen	-	0.08
68	4-Quinololinol,2-methyl- β -2-Methy	-	0.03
69	2-(methylthio)-Ethanamine	-	0.03
70	(Z,Z)- α -Farnesene	0.17	-
71	Ethyl(isopropyl)propylborane	0.14	-
72	Phthalic acid, cyclobutyl tridecyl est	0.57	0.12
73	4-Hydroxytranylcypramine	0.11	-
Total		100	100

3.2. Larvicidal Activity of Essential Oil of *Laggera pterodonta*

The mortality of *Anopheles gambiae* against different concentration of essential oil of *Laggera pterodonta* is shown in Figure 1. The mortality ranges from 26.7% and 35% at concentration of 200 mg/L after 24 h and 48 h to 100% at concentration of 2000 mg/L. No mortality was observed in the control. There was significant difference ($p < 0.05$) in mortality of larvae amongst the treatments.

3.3. Larvicidal Activity of Essential Oil of *Laggera aurita*

The result of the larval mortality of the *Anopheles gambiae* on exposure to different doses of the essential oil of *Laggera aurita* is shown in Figure 2. At the concentration of 2000 mg/L, 100% mortality was recorded while 93.5% and 98.5% mortalities were recorded at concentration of 1000 mg/L after 24 h and 48 h respectively. The lowest mortality of 6.5% and 11.7% was observed at the dose of 200 mg/L after 24 and 48 h respectively. No mortality was observed in the control. There was significant difference ($p < 0.05$) in mortality of larvae amongst the treatments.

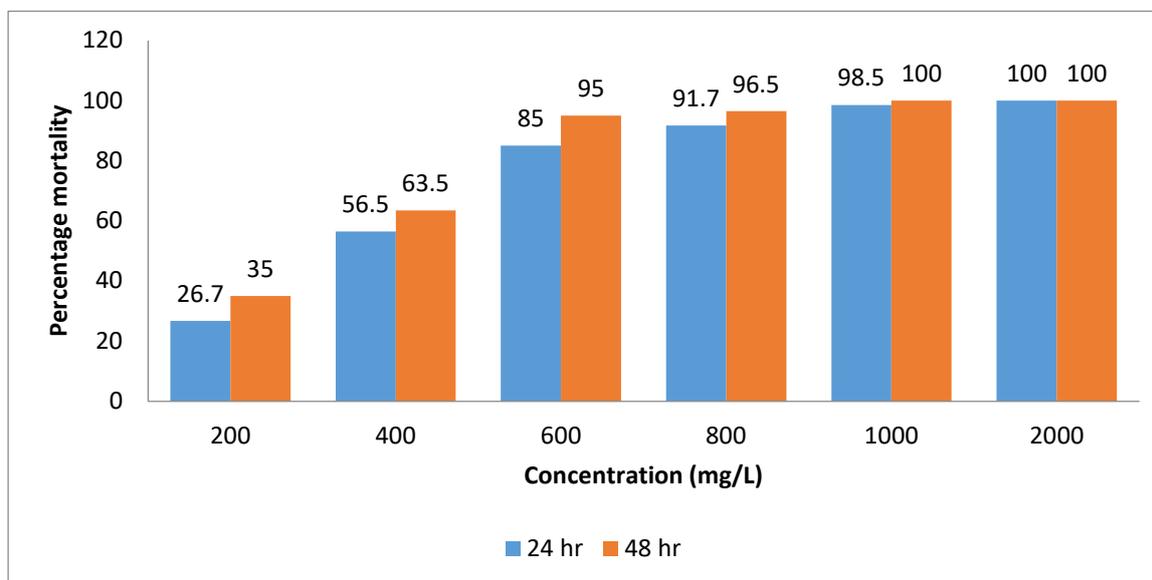


Figure 1. Larvicidal activity of essential oil of *Laggera pterodonta*.

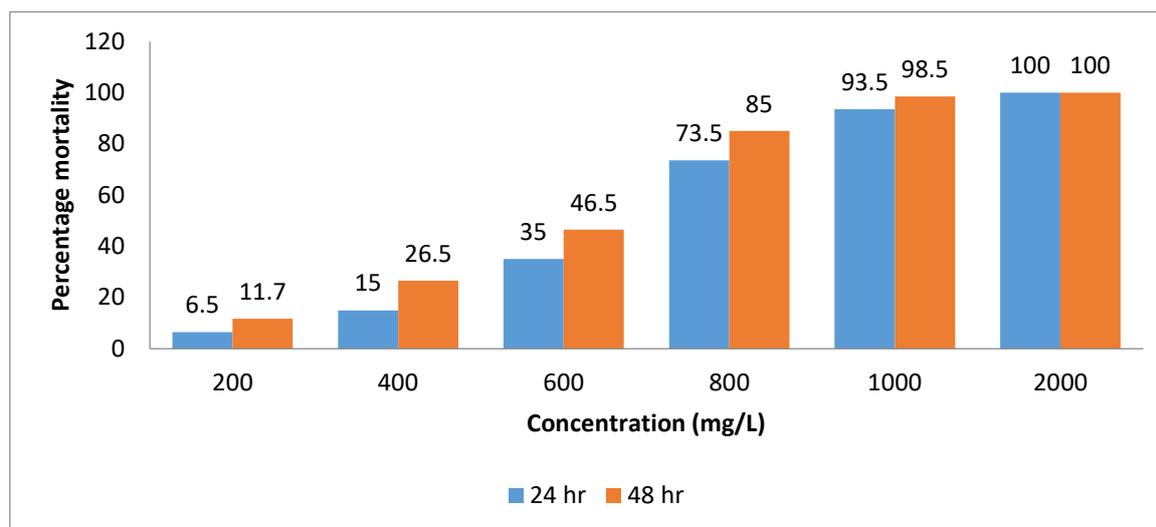


Figure 2. Larvicidal activity of essential oil of *Laggera aurita*.

3.4. Lethal Concentration of Essential Oils against Larvae of *An. gambiae*

The lethal concentration of essential oils against larvae of *An. gambiae* is shown in Table 2. Essential oil of *L. pterodonta* was more toxic to the larvae of *An. gambiae* with LC₅₀ of 418 and 404 mg/L after 24 and 48 h respectively while essential oil of *L. aurita* had LC₅₀ values of 688 and 642 mg/L after 24 and 48 h respectively.

Table 2. Lethal concentration of essential oils against larvae of *An. gambiae*.

Extracts	LC ₅₀ (LB-UB)mg/L		LC ₉₀ (LB-UB)mg/L	
	24 h	48 h	24 h	48 h
<i>Laggera pterodonta</i>	418 (284–503)	404 (283–475)	753 (655–892)	628 (543–762)
<i>Laggera aurita</i>	688 (603–740)	642 (485–704)	973 (896–1139)	870 (804–1059)

LB = lower bound, UB = upper bound.

4. Discussion

Essential oils are defined as any volatile oil (s) that have strong aromatic components and that give distinctive odour, flavor or scent to a plant (Asgar et al., 2013). Essential oil of a plant is a complex mixture that may contain approximately 30–100 different constituents but certain components will be present in larger quantities (Gangwar and Tiwari, 2017). The composition analysis of the two *Laggera* essential oils revealed several different components. However, some of the components of these essential oils have been reported to have insecticidal, repellent and insectistatic activity against various insects. Thus might have been acting in synergistic way to bring about the mosquitocidal effect observed in the study. For instance, Caryophyllene also known as β-Caryophyllene detected as one of the major constituent of the oils of *Laggera aurita* and *Laggera pterodonta* in this study could have contributed to the toxicities since it was reported to exhibit larvicidal activity against *Aedes aegypti* (Ali et al., 2014; Doria et al., 2010). Its oxygenated form caryophyllene oxide detected only in *L. pterodonta* oil was reported to have larvicidal activity against different species of mosquitoes (Zhu and Tian, 2013; Chintem and Nzalibe, 2015b; Ali et al., 2014; Zhu et al., 2015). α-Caryophyllene detect in both oils in the present study was reported by Guo et al. (2017) as one of the major component of the essential oil of *L. pterodonta* that exhibited toxicity and repellency against adult *Liposcelis bostrychophila* (stored product pest). It can therefore be deduced that these metabolite could have worked in a synergistic way to bring about the mortality of the larvae observed in this study.

The essential oils of both *L. pterodonta* and *L. aurita* showed very high larvicidal activity with lethal concentration that could kill 50% of the target larvae as 418 and 408 mg/L for *L. pterodonta* and

688 and 642 mg/L for *L. aurita* at 95% confidence interval. Ombito et al. (2014) reported the larvicidal activity of the essential oil of *Zanthoxylum gilleti* against *Anopheles gambiae* and observed LC₅₀ 57.73 mg/mL. The essential oil of

The essential oil of *L. pterodonta* in this study was significantly ($p < 0.05$) more toxic to mosquito than the oil of *L. aurita*. The higher mosquitocidal activity of *L. pterodonta* essential oil in this study could be attributed to the presence of higher percentage of compounds such as α -caryophyllene, γ -terpinene, 4-carvomenthenol, linalool and (+)-4-carene which are known for their potential insecticidal activity. In addition minor compounds of *L. pterodonta* such as limonene, caryophyllene oxide and sabinene have also been reported for their potential insecticidal activity and might have contributed to the higher toxicity of *L. pterodonta*.

5. Conclusions

In this study, the result indicates that the essential oil of *Laggetta pterodonta* and *L. aurita* holds great promise as potential larvicides. Such findings also offer an opportunity for developing newer more selective, biodegradable and natural mosquitocidal compounds.

Acknowledgments: The authors are grateful to the National Institute of Pharmaceutical Research and Development (NIPRD) for availing their laboratory for the research and the technical support provided by Malam Ibrahim Muazzam.

References

1. Ali, A.; Tabanca, N.; Kurkcuoglu, M.; Duran, A.; Bylthe, E.K.; Khan, I.A.; Baser, K.H. Chemical composition, larvicidal and biting deterrent activity of essential oils of two subspecies of *Tanacetum argenteum* (Asterales: Asteraceae) and individual constituents against *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* **2014**, *51*, 824–830.
2. Doria, G.A.A.; Silva, W.J.; Carvalho, G.A.; Alves, P.B.; Cavalcanti, S.C.H. A study of larvicidal activity of two *Croton* species from northeastern Brazil against *Aedes aegypti*. *Pharm. Biol.* **2010**, *48*, 615–620.
3. Chintem, W.D.; Nzeliibe, C.H. Larvicidal activity of leaf extracts and purified fractions of *Ocimum gratissimum* against *Culex quinquefasciatus* mosquito larvae. *Int. J. Sci. Res.* **2015**, *4*, 12.
4. Gangwar, P.; Tiwari, S.N. Insecticidal Activity of *Curcuma longa* Essential Oil and its Fractions against *Sitophilus oryzae* L. and *Rhyzopertha dominica* F. (Coleoptera). *Int. J. Pure Appl. Biosci.* **2017**, *5*, 912–921.
5. Gillies, M.T.; De Meillon, B. The Anophelinae of Africa South of the Sahara (Ethiopian Zoogeographical Region). *S. Afr. Inst. Med Res. Johannesbg.* **1968**, *2*, 1–343.
6. Guo, S.S.; Zhang, W.J.; You, C.X.; Liang, J.Y.; Yang, K.; Geng, Z.F.; Du, S.S.; Wang, C.F. Chemical Composition of Essential Oil Extracted from *Laggetta pterodonta* and its Bioactivities against Two Stored Product Insects. *J. Food Process. Preserv.* **2017**, *41*, 21–24.
7. Langsi, D.J.; Nukenine, E.N.; Fokunang, C.N.; Goudoungon, J.W.; Kosini, D.; Fotso, T.G.; Tchinda, G.; Agbor, G. Evaluation of the insecticidal properties of fractionated extracts of *Ocimum canum* and *Laggetta pterodonta* on stored maize against the infestation of *Sitophilus zeamisi* (Coleoptera: Curculidae). *Int. J. Agron. Agric. Res.* **2017**, *10*, 41–50.
8. Maharaj, R.; Maharaj, V.; Crouch, N.R.; Bhagwandin, N.; Folb, P.I.; Pillay, P.; Gayaram, R. Screening for adulticidal bioactivity of South African plants against *Anopheles arabiensis*. *Malar. J.* **2011**, *10*, 1–5.
9. Mudalungu, C.M.; Matasyoh, J.C.; Vulule, J.M.; Chepkorir, R. Larvicidal compounds from the plant *Fagaropsis angolensis* leaves against malaria vector (*Anopheles gambiae*). *Int. J. Malar. Res. Rev.* **2013**, *1*, 1–7.
10. Ohimain, E.I.; Angaye, T.C.N.; Basse, S.E. Comparative larvicidal activities of the leaves, bark, stem and root of *Jatropha curcas* (Euphorbiaceae) against malaria vector *Anopheles gambiae*. *Sky J. Biochem. Res.* **2014**, *3*, 29–32.
11. Okhale, S.E.; Ugbabe, G.E.; Oladosu, P.O.; Ibrahim, J.A.; Egharevba, H.O.; Kunle, O.F.; Elisha, E.P.; Chibuikwe, A.J.; Ettah, U.O. Chemical constituents and antimicrobial activity of the leaf essential oil of *Ixora coccinea* L. (Rubiaceae) collected from North Central Nigeria. *Int. J. Bioassays* **2018**, *7*, 5630–5637.
12. Ollengo, M.A.; Vulule, J.M.; Matasyoh, J.C. Isolation, structure elucidation and larvicidal activity of *Laggetta alata* extracts. *Res. J. Pharmacogn. Phytochem.* **2016**, *8*, 153–164.

13. Ombito, O.J.; Matasyoh, C.J.; Vulule, M.J. Chemical composition and larvicidal activity of *Zanthoxylum gillettii* essential oil against *Anopheles gambiae*. *Afr. J. Biotechnol.* **2014**, *13*, 2175–2180.
14. Omoregie, E.H.; Oluyemisi, K.F.; Koma, O.S.; Ibumeh, O.J. Chemical Constituents of the Essential Oil of *Laggera pterodonta* (DC.) Sch. Bip. from North-Central. *Niger. J. Appl. Pharm. Sci.* **2012**, *2*, 198–202.
15. Singh, S.P.; Mittal, P.K. Mosquito repellent and oviposition deterrent activities of *Laggera aurita* plant extract against malaria vector *Anopheles stephensi*. *Entomol. Appl. Sci. Lett.* **2015**, *2*, 18–22.
16. World Health Organization. Malaria Fact Sheet No 94. 5pp. 2015.
17. Zhu, L.; Tian, Y.J. Chemical composition and larvicidal activity of essential oil of *Artemesia gilvoescens* against *Anopheles anthropophagus*. *Parasitol. Res.* **2013**, *112*, 1137–1142.
18. Zhu, S.; Liu, X.C.; Liu, Z.L.; Xu, X. Chemical composition of *Salvia plebeian* R. Br. essential oil and its larvicidal activity against *Aedes aegypti* L. *Trop. J. Pharm. Res.* **2015**, *14*, 831–836.

Publisher’s Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



© 2020 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).