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Proceeding Paper

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

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Abstract: The role of plants as sources of biologically active entities cannot be overstated. Plants belonging to different families with mosquitocidal activity have been discovered in established ethnobotanical and laboratory-based studies. Biological control of mosquitoes using botanicals remains the safest and most environmentally sound alternative to chemical control. The essential oil of two Nigerian *Laggera species*, *L. pterodonta* and *L. aurita*, were investigated with regard to their ability to kill the fourth instar larvae of the malaria vector, *Anopheles gambiae*. The WHO protocol was adopted for the larvicidal bioassay. Three replicates comprising 20 larvae each were exposed to various concentrations of the essential oil. Larval mortality was observed after 24 and 48 h, respectively. The results show that mortality increased with an increase in concentration and period of exposure. The essential oil of *L. pterodonta* was found to be the most effective, with LC_{50} values of 418 and 404 mg/L after 24 and 48 h, respectively, while the essential oil of *L. aurita* recorded LC_{50} value of 688 and 642 mg/L after 24 and 48 h, respectively. The GC-MS results reveal that 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 a higher percentage of compounds such as ɤ-terpinene and 4-carvomenthenol with larvicidal activity. The results suggest that the essential oil of the plants have the potential to be used as an eco-friendly approach for the control of mosquitoes.

Keywords: *Laggera pterodonta*; *L. aurita*; essential oil; larvicides; *Anopheles gambiae*

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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 [1]. 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 been shown to have various levels of bioactivity against different developmental stages (larval, pupal and adult) of mosquitoes [2]. Larvicide is more effective than the control of adults due to the limited area of larvae movement compared to free flying adults [3]. However, intensive use of synthetic insecticides in mosquito control programs has created widespread resistance [4], undesirable effects on other insects and negative impacts on the environment [3]. These problems have highlighted the need for exploration and the 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 modes of action and

are biodegradable. In fact, many researchers have reported on the effectiveness of plant essential oils against mosquito larvae, and recent examples include studies [3,4].

The genus *Laggera* belongs to the family Asteraceae and consists of about 20 species. Plants in the genus *Laggera* are aromatic and are mostly found growing as weeds in Africa and Asia. Previous research has shown the efficacy of the plants in the genus *Laggera* against various insects; this includes insecticidal activity against maize weevil [5], mosquito larvicidal activity [6], as well as mosquito repellent and oviposition deterrent [7]. *Laggera pterodonta* and *Laggera aurita* are the two most common species found growing in Nigeria. They are used mainly as antispasmodics, diuretics, laxatives and antidysentrics [7].

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 a voucher specimen number of NIPRD/H/6977and NIPRD/H/6978 for *Laggera aurita* and *Laggera pterodonta*, respectively, by a botanist at the Medicinal Plant Research and Traditional Medicine (MPR and TM) Department of the National Institute of Pharmaceutical Research and Development (NIPRD).

2.2. Essential Oil Extraction

The essential oils were extracted at the MPR and TM Department (NIPRD). Each fresh plant (*Laggera pterodonta* and *Laggera aurita*) was sorted and cut into small pieces that were meant to increase the surface area during the heating process. A two-liter round-bottomed flask was packed with the plant pieces (including water) connected to Clevenger apparatus and placed on a heating mantle. The hydro-distillation process was carried out for about six hours according to the British pharmacopoeia [8]. 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 bioassays were carried out [9].

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

The essential oils were analyzed 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 a HP-5ms fused silica capillary with a 5% phenyl-methylpolysiloxane stationary phase, with a length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with a flow rate of 1.61 mL/min. The program used for gas chromatography had an oven temperature of 60–160 °C at a rate of 15 °C/min, which was 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. A diluted sample (1/100 in hexane, *v*/*v*) of 1.0 µL was injected using autosampler and in the split mode with a ratio of 20:80. Individual constituents were identified by comparing their mass spectra with the NIST Mass Spectral Library (NIST 08). The percentages of each component are reported as raw percentages based on the total ion current without standardization [10].

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 [11]. It is important to note

that *Anopheles* larvae were identified by the absence of siphon and they stay parallel to surface of water. The 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 adults emerged and were found hanging on the net. The adult mosquitoes were identified up to species level by consulting keys [11].

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 (2005) [12] protocol. Twenty 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 the control was run simultaneously. Mortality was recorded after 24 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 analyzing the data. Probit analysis was used to determine the LC_{50} and LC_{90} 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 *Laggera pterodonta* and *Laggera aurita* are shown in Table 1. A total of 44 chemical components were identified and quantified from the essential oil of *Laggera aurita*. The major components were benzene, 2-tert-butyl-1,4-dimethoxy (25.24%), caryophyllene (12.25%) and ɤ-terpinene (8.92%). On the other hand, a total of 45 constituents were identified from the essential oil of *Laggera pterodonta*. The major components were ɤ-terpinene (12.72%), benzene,2-tert-butyl-1,4-dimethoxy (12.66%), (+)-4-carene (10.40%), 4-carvomenthenol (9.22%), and ɤ-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 the following eighteen compounds in common; α–phellandrene, (+)-4-carene, o-cymene, ɤ-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 *Laggera pterodonta and Laggera aurita* essential oil by GC-MS.

ID	Compound	Percentage (%)	
		L. pterondata	*L. aurita*
1	Bicylo[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 –Methylmercaptoeethylamine	-	0.10
12	α –Limonene	0.93	-
13	Hyacinthin	0.68	-
14	ɤ-Terpinene	12.72	8.92
15	β –Linalool	2.25	0.43
16	2-Carene	3.46	1.38

Table 1. *Cont.*

ID	Compound	Percentage (%)	
		L. pterondata	*L. aurita*
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)-Caren-4-ol, aceacetic acid es	0.53	-
21	6-Methylenebicyclo[3.1.0]hexane	0.18	-
22	1,4-Dimethyl-delta-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	ɤ-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 peroxynitrate	-	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-metho	0.18	-
44	Cyclohexane, butylidene	0.20	-
45	ɤ -Eudesmol	8.67	-
46	2-Isopropenyl-1,3-dimethylcyclopen	-	1.34
47	δ-Cadinol,(-)-	2.03	-
48	α –Cadinol	-	5.29
49	ɤ-Eudesmol	1.42	-
50	α –Muurolene	-	0.30
51	tau-Muurolol	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-methylcylohexane	-	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-Quinolinol,2-methyl-$$ 2-Methy	-	0.03
69	2-(methylthio)-Ethanamine	-	0.03
70	(Z,Z)-α-Famesene	0.17	-
71	Ethyl(isopropyl)propylborane	0.14	-
72	Phthalic acid, cyclobutyl tridecly est	0.57	0.12
73	4-Hydroxytranylcypromine	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 a concentration of 200 mg/L after 24 h and 48 h to 100% at a concentration of 2000 mg/L. No mortality was observed in the control. There was a significant difference ($p < 0.05$) in the mortality of larvae amongst the treatments.

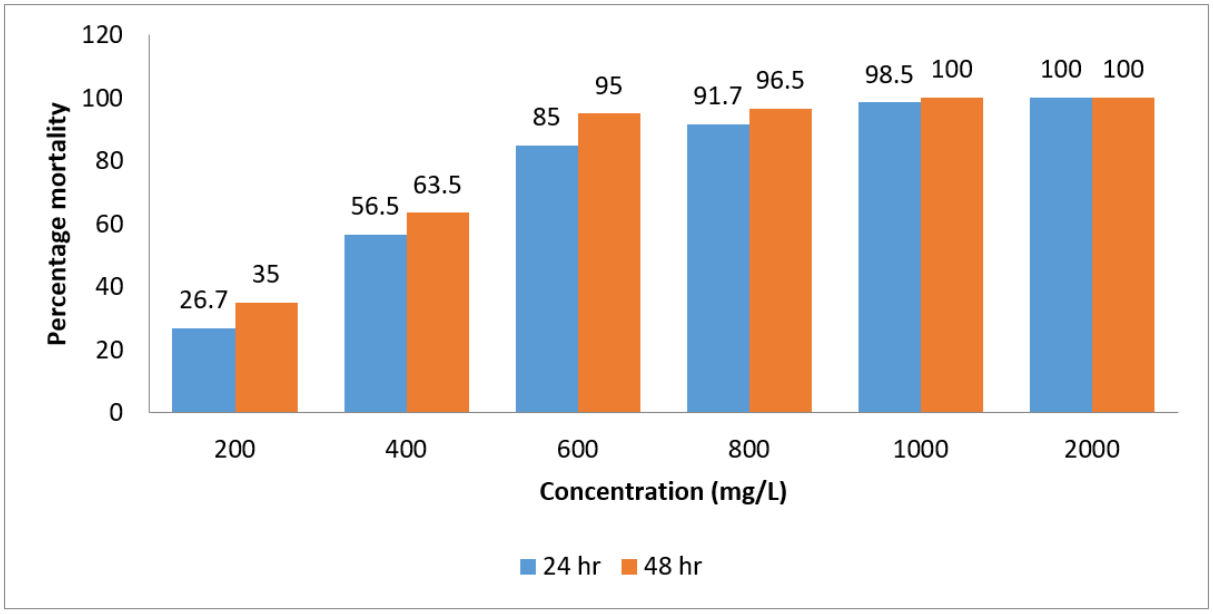

Figure 1. Larvicidal activity of essential oil of *Laggera pterodonta*.

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 concentrations of 1000 mg/L after 24 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 for the control. There was significant difference ($p < 0.05$) in the mortality of larvae amongst the treatments.

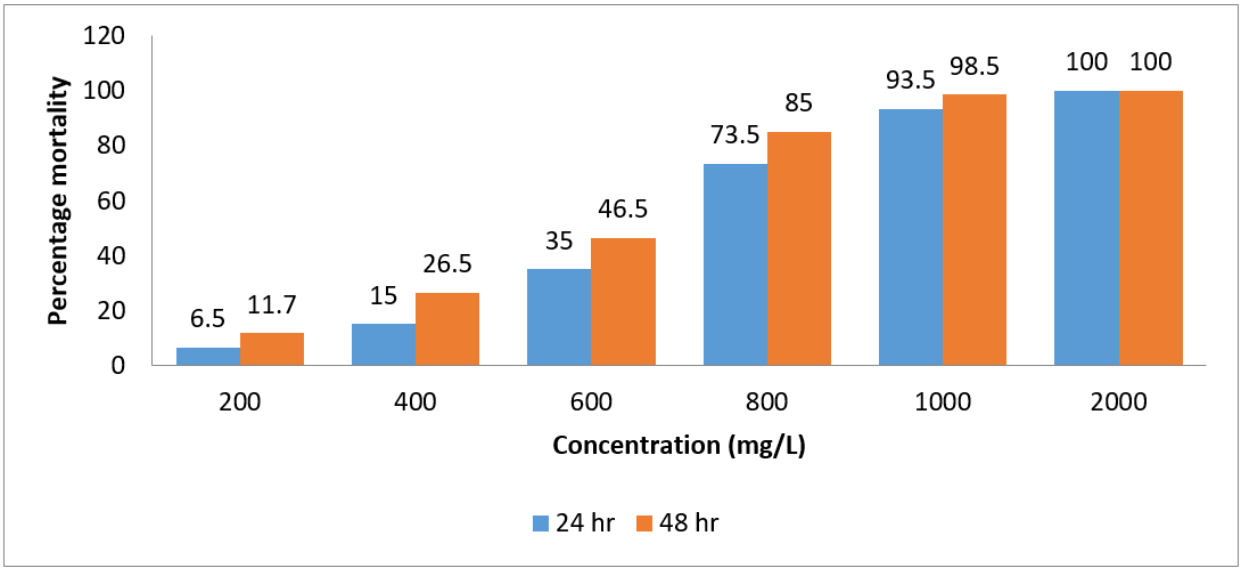

Figure 2. Larvicidal activity of essential oil of *Laggera aurita*.

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 LC_{50} of 418 and 404 mg/L after 24 and 48 h, respectively, while essential oil of *L. aurita* had LC_{50} 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
Laggera pterodonta	418 (284–503)	404 (283–475)	753 (655–892)	628 (543–762)
Laggera aurita	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 oils that have strong aromatic components and that give distinctive odor, flavor or scent to a plant [13]. An 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 [14]. 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. This 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 constituents 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* [15,16]. Its oxygenated form caryophyllene oxide detected only in *L. pterodonta* oil was reported to have larvicidal activity against different species of mosquitoes [15,17–19]). α-caryophyllene detected in both oils in the present study was reported [20] as one of the major components 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 metabolites 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 a lethal concentration that could kill 50% of the target larvae of 418 and 408 mg/L for *L. pterodonta* and 688 and 642 mg/L for *L. aurita* at a 95% confidence interval, respectively. A study [4] reported the larvicidal activity of the essential oil of *Zanthoxylum gilleti* against *Anopheles gambiae* and observed LC_{50} 57.73 mg/mL.

The essential oil of *L. pterodonta* in this study was significantly ($p < 0.05$) more toxic to mosquitos 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 a higher percentage of compounds such as α-caryophyllene, ɤ-terpinene, 4-carvomethenol, 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 *Laggera 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.

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