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Chemical Composition, Larvicidal and Adult Emergence Inhibition Activities of *Balanites aegyptiaca* Del. Seed and *Aristolochia albida* Duch. Root Extracts against Malaria Vector, *Anopheles gambiae* Giles

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Abstract

Background: *Anopheles gambiae* is enemy number one of mankind in Africa and particularly in Cameroon due to its ability of transmitting malaria which is the deadliest disease in this part of the world. Synthetic insecticides have been used to control malaria vectors but they have negative effects on non-target organisms and are environmentally unfriendly. Control of mosquitoes at larval stages using phytochemicals is currently the leading tool to reduce the mosquito population and so the reduction of malaria transmission rates. Therefore, the present study was to evaluate the phytochemical contents, larvicidal and adult emergence inhibition activities of *Balanites aegyptiaca* seed and *Aristolochia albida* root solvents extracts against *Anopheles gambiae* larvae. **Methods:** The World Health Organization standard protocols were followed for the different bioassays. Concentrations ranging from 500 - 2000 ppm for larvicidal and 500 - 1500 ppm for IGRs were used. **Results**: Both plants showed the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids and oils except the absence of phenols in *Aristolochia albida*. The larvicidal activity of *Aristolochia albida* extracts showed that hexane and methanol fractions were the most active killing relatively all exposed larvae with the LC_{50} values of 420.1 and 453 ppm, respectively. The same observation was made in hexane fraction from *Balanites aegyptiaca* (LC_{50} = 588 ppm). The insect growth inhibitory activity of *Aristolochia albida* extracts proved that hexane and methanol fractions relatively caused a 100%

inhibition in the mosquito development, recording the EI_{50} values of 482.4 and 555.6 ppm, respectively. The same trend was observed with *Balanites aegyptiaca* hexane fraction registering better EI_{50} of 623.9 ppm. **Conclusions:** Our findings demonstrate that *Balanites aegyptiaca* seed and *Aristolochia albida* seed extracts are rich in phytochemicals capable of killing mosquito larvae and disrupting mosquito larval development. This could contribute to the control of mosquito populations and improved management of malaria.

Keywords

Balanites aegyptiaca, *Aristolochia albida*, *Anopheles gambiae*, Larvicidal Activity, IGRs, Vector Control

1. Introduction

According to world malaria reports 2021, there were an estimated 227 million malaria cases globally in the 85 malaria endemic countries in 2019. In 2020, a year after the COVID-19 pandemic and service disruptions, the estimated number of malaria cases rose to 241 million cases (additional 14 million cases compared with 2019) [1]. In Africa, estimated malaria cases increased from 213 million to 228 million, and deaths from 534,000 in 2019 to 602,000 in 2020. This region accounted for about 95% of cases and 96% of deaths globally; 80% of all deaths in this region are among children aged less than 5 years [1]. Cameroon is among the eleven African countries where malaria mortality and morbidity are the highest, accounting for 70% of the global estimated case burden and 71% of global estimated deaths [1]. *Anopheles gambiae* is the predominant species of mosquito responsible for transmission of malaria and *Plasmodium falciparum* is the principal parasite species [1]. The risk factors for malaria transmission are the presence of water-retaining containers in and around households, lack or poor draining systems, lack of knowledge of mosquitoes biology, inappropriate disposal of waste, non-use of LLINs, absence of window and door nets, insect repellent spray, mosquito coil, mosquito repellent body cream, mosquito candle, mosquito spray, poor management of toilets, etc. [1] [2].

The current methods for the treatment of malaria cases rely on the use of artemether, amodiaquine, artemisinin, artesunate, clindamycline, chloroquine, dihydroartemisinin, mefloquine, piperaquine, primaquine, pyronaridine, quinine, artemisinin-based combination therapy (artemether + lumefantrine, artesunate + amodiaquine, artesunate + mefloquine, dihydroartemisinin + piperaquine and artesunate + sulfadoxine-pyrimethamine) and intermittent preventive treatment [1] [2]. However, the best approach for the fight against this deadly disease is its vector control which relies principally on integrated vector management approaches such as targeting adult stages using long-lasting insecticide treated nets and indoor residual spraying, clean environment and reduction of immature mosquito stages [1]. In Cameroon, the current strategies to reverse the

trend are based on the mass distribution of long-lasting insecticidal nets, the intermittent preventive treatment of malaria in pregnancy, the seasonal malaria chemoprevention in infants and the free of charge systematic treatment of uncomplicated malaria in under-five children [2]. Targeting mosquitoes at larval stage seems to produce better results because of the interruption of their life cycle and so their population dynamics [1]. To this effect, synthetic mosquito larvicides such as pyrethroids, organochlorides, organophosphates and carbamates or insect growth regulators (IGRs) such as chitin synthesis inhibitors and juvenoids have been found very effective against different mosquito species [3]. However, over the years, they showed negative impacts on non-target organisms and vectors managed to develop resistance against them [4]. This prompted researchers to look for alternative candidates. Plants have been considered as alternative sources for synthetic mosquito larvicides and IGRs largely because they constitute a potential source of bioactive secondary substances that have been perceived by the general public as relatively safe and with less risk to the environment and with minimal impacts to animal and human health, easily biodegradable and available for resource-poor farmers in developing countries [5] [6]; among those plants are *Aristolochia albida* and *Balanites aegyptiaca*.

Balanites aegyptiaca Del. (Zygophyllaceae), known as "desert date," is spiny shrub or tree up to 10 m tall, widely distributed in dry land areas of Africa and South Asia. It is traditionally used in treatment of various ailments such as malaria, wounds, dysentery, jaundice, stomach aches, intestinal worm infection, syphilis, epilepsy, constipation, diarrhea, hemorrhoid, asthma, and fever. It contains protein, lipid, carbohydrate, alkaloid, saponin, flavonoid and organic acid [7]. Elsewhere, fruit kernel of *B. aegyptiaca* extract has been found to have mosquito larvicidal activity against *An. arabiensis*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae [7].

Aristolochia albida Duch. (Aristolochiaceae) is a wild herb/shrub commonly used in traditional medicine. It has multiple applications and virtues; it is recommended for ovarian failure, healing diuretic, analgesic, anti-inflammatory, anti-cancer, especially in case of sclerosis, uterine and nasal cancer. The powder of roots with salted butter is used to treat skin infections and gangrene [8]. The plant is rich in total phenols, tannins and flavonoids and is said to have antioxidant properties [9]. There is no information about the activity of this plant against mosquito.

The present study was to evaluate the phytochemical composition, the larvicidal and adult emergence inhibition activities of *Balanites aegyptiaca* seed and *Aristolochia albida* root solvents extracts against larvae of malaria vector mosquito, *An. gambiae*.

2. Materials and Methods

2.1. Collection of Plant Materials

The fresh roots of *Aristolochia albida* were harvested in August 2020 (7:00 - 9:00

GMT) from Doukoula, Mayo-Danay Division, Far-North Region, Cameroon (latitude 9˚59'384"N˚, longitude 15˚33'833"E˚, altitude of 330 masl) and washed with distilled water. The dried fruits of *Balanites aegyptiaca* were bought from Garoua "Grand-marché", located in the North region, Cameroon. They were identified for confirmation by Dr Gilbert Haïwa, a botanist from the University of Maroua. Roots were dried at room temperature and ground in powdered form using mortar and pestle until the powder passed through a 0.4 mm mesh sieve. Due to their extremely hard nature, the dried fruits of *B. aegyptiaca* were broken manually using a harmer and a stone and the seeds were collected. The seeds were then well ground using a groundnut grinding machine. The machine was first of all dismantled, thoroughly washed using soap and brush and finally rinsed with distilled water. The ground plant materials were stored in opaque containers inside a refrigerator at −4˚C until needed.

2.2. Extraction and Fractionation of Plant Materials

The method adopted by Danga *et al.* [10] was used for the extraction and fractionation processes. 800 g of each ground material were extracted by cold maceration in methanol in the laboratory of Institute of Medical Research and Medicinal Plants Studies (IMPM), Yaoundé, Cameroon. The maceration process was repeated thrice for maximal extraction. The methanol crude extract was then collected and concentrated almost to dryness under vacuum at 40˚C using rotary evaporator. The methanol crude extract was first mixed with silica gel (60 - 200 mesh size) and sequentially fractionated in hexane, ethyl acetate and methanol following their order of polarity. All the fractions were filtered many times adding fresh solvent until clear phase was obtained before passing to the next solvent using Whatman N˚. 1 filter paper. The same rotary evaporator was used to concentrate the fractions at 40˚C ± 5˚C. The crude extracts and fractions were stored in refrigerator at −4˚C until needed.

2.3. Phytochemical Analysis

The phytochemical analysis of *A. albida* root and *B. aegyptiaca* seed extracts and fractions was carried out following the standard protocols [11] [12].

2.4. Source of *An. gambiae* Larvae

Due to the fact that the small-scale and the large-scale field trials are the next steps of this study, ideally, the larvae of *An. gambiae* were collected from the field. This is because they were adapted to their natural milieu and so to the chemical and environmental constraints where they were living. They were collected from stagnant water in the blocked gutter at Barmary quarter in Garoua, North Region of Cameroon (9˚97'091"N˚, 13˚33'617"E˚, 220 masl) and identified by Dr Simon Pierre Yinyang Danga, medical entomologist, University of Garoua, in February 2021. Larvae were kept in plastic trays containing well water in the laboratory. Larvae were fed with a diet containing crayfish and biscuit in 3:1

ratio, respectively.

2.5. Preparation of Stock Solutions, Test Concentrations and Larvicidal bioassays

The WHO standard procedure [3] was respected to determine the bio-efficacy of the plant extracts and fractions against *An. gambiae* larvae. Stock solutions for each extract were made by weighing 1g of each extract and using Tween 80 (Polyoxyethylene sorbitan monooleate) as emulsifier to ease the dissolution of the extracts in water. The stock solutions were made up of diluted extract and well water making an aliquot of 100 ml for each extract in each glass beaker.

From these stock solutions, serial dilutions were made by using 5 ml and 10 ml syringes with the addition of tap water and making four concentrations ranging from 500 to 2000 ppm. For comparison, a commercial insecticide, RAMBO® (Permethrin 0.6%) powder (500 ppm), bought from Garoua market, was used as positive control. 1 ml of Tween 80 in 99 ml of tap water was used as negative control for each replicate and extract.

For the larvicidal bioassay, 20 fourth instar larvae of *An. gambiae* were subjected to each 150 ml glass beaker containing 100 ml of test solution and mortality was recorded 24 h after exposure under room temperature with relative humidity of 22˚C - 39˚C and 23% ± 2% and photoperiod of 12L:12D. No food was provided. There were three replicates for each extract and concentration.

For insect growth regulating (IGR) activity, the same procedure as for larvicidal activity was used with some exceptions. Due to very high larval mortality in some extracts, five lower concentrations were made ranging from 500 to 1500 ppm, third instar larvae were rather used. Test and control beakers were covered with netting to prevent successfully emerged adults from escaping into the environment. Food was provided at two-day intervals until mortality counts were made. Mortality or survival was counted every other day until the complete emergence of adults. The test containers were held at 21˚C - 41˚C and for a photoperiod of 12L:12D. At the end of the observation period, the impact was expressed as IE% based on the number of larvae that did not develop successfully into viable adults. In recording IE% for each concentration, moribund and dead larvae and pupae, as well as adult mosquitoes not completely separated from the pupal case, were considered as "affected". Successfully emerged adults were counted from the empty pupal cases. The experiment stopped when all the larvae or pupae in the controls were dead or emerged as adults.

2.6. Statistical Analysis

For larvicidal activity, wherever mortality in negative control reached 5%, Abbott's formula [13] was applied to correct it. Mortality data were subjected to ANOVA procedure using SPSS 17.0. Student-Newman-Keuls test ($p = 0.05$) was applied for mean separation. A logarithmic transformation [$\log_{10}(x + 1)$, where x = content in %] was performed before regression analysis. Probit analysis [14]

was applied to determine lethal concentrations causing 50% (LC_{50}) and 90% (LC_{90}) mortality of larvae 24 h post-exposure as well as their fiducial limits.

For IGR activity, data from all replicates of each concentration were combined. Mean emergence inhibition was calculated on the basis of the number of exposed larvae using ANOVA procedure as for larvicidal bioassay. The overall emergence of adults reflected activity. IE% was calculated using the following formula: IE (%) = 100 − (T × 100/C), where T = emergence in treated batches and C = emergence in the control. If adult emergence in the control was less than 80%, the test was discarded and repeated. Where the percentage was between 80% and 95%, the data were corrected using Abbott's formula [13]. A logarithmic transformation [$\log_{10}(x + 1)$, where x = content in %] was performed before regression analysis. IE values obtained at each concentration were subjected to probit regression analysis [14] to determine IE_{50} and IE_{90} values using SPSS 17.0 software.

3. Results

The yields of crude extract and fractions from *Balanites aegyptiaca* and *Aristolochia albida* presented in Table 1 revealed that both plants had relative identical yield of methanol crude extract. However, *Aristolochia albida* harvested better yield of n-hexane and ethyl acetate fractions.

The qualitative phytochemical analysis of *Balanites aegyptiaca* methanol crude extract (Table 2) uncovered the presence of alkaloids, flavonoids, saponins, tannins, phenols, terpenoids and oils. The same compounds were equally present in n-hexane fraction. However, only alkaloids, tannins and phenols were present in ethyl acetate fraction and alkaloids and saponins were found in methanol fraction. Oils were only present in methanol rude extract and hexane fraction.

The results of the qualitative phytochemical analysis of *Aristolochia albida* revealed the presence of alkaloids, flavonoids, saponins, tannins and terpenoids in methanol crude extract as well as in all resulted solvent fractions except in methanol fraction where flavonoids were absent. Oils were found only in methanol rude extract and hexane fraction (Table 3).

Table 1. Yields of crude extracts and their fractions from *Balanites aegyptiaca* and *Aristolochia albida*.

Extract/fractions	Yields in %	
	Balanites aegyptiaca	*Aristolochia albida*
Methanol crude extract	6.4	5.6
n-hexane fraction	2.5	13.5
Ethyl acetate fraction	1.6	36.5
Methanol fraction	76.4	46.2

Table 2. Phytochemical constituents of *Balanites aegyptiaca.*

Extracts	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Steroids	Terpenoids	Oils
M. cr. ex.	+	+	+	++	+	−	+	+
Hex. fr.	+	+	+	++	+	−	++	+++
Eth.a. fr.	+	−	−	+	+	−	−	−
Meth. Fr.	+	−	++	−	−	−	−	+

+: present; −: absent.

Table 3. Phytochemical constituents of *Aristolochia albida.*

Extracts	Alkaloids	Flavonoids	Saponins	Tannins	Phenols	Steroids	Terpenoids	Oils
M. cr. ex.	+	+	+++	+	−	−	+	+
Hex. Fra.	+	+	++	+	−	−	+	+
Eth. a. fr.	+	+	+	+	−	−	+	−
Meth. fr.	+	−	+	+	−	−	+	−

+: present; −: absent.

The larvicidal activity of *Aristolochia albida* crude extract and fractions against *Anopheles gambiae* showed that hexane and methanol fractions as well as methanol crude extract were the most active killing relatively all exposed larvae with the LC_{50} values of 420.1, 453 and 588 ppm, respectively (Table 4).

All fractions and crude extract from *Balanites aegyptiaca* were toxic against *Anopheles gambiae* larvae (Table 5). Toxicity of fractions and crude extract were relatively identical with hexane and crude extract exhibiting more activity than the others (LC_{50} = 588 and 642.6 ppm, respectively). No larva was found alive in beakers where the conventional Permethrin was added.

The insect growth inhibitory activity of methanol crude extract and fractions of *A. albida* tested against the larvae of *A. gambiae* is presented in Table 6. Hexane and methanol fractions relatively caused a 100% inhibition in the mosquito development, recording the EI_{50} values of 482.4 and 555.6 ppm, respectively. Almost all deaths were recorded at larval stage with white soft body, only few succeeded in turning into pupal and adult stages at lower concentrations.

Table 7 shows the growth regulatory activity of *B. aegyptiaca* extracts against *Anopheles gambiae*. Data suggest that the activity was dose-dependent, increasing with the increase of concentrations. All extracts globally recorded the same growth inhibitory activity with hexane having better EI_{50} of 623.9 ppm followed by methanol crude extract (EI_{50} = 921.1 ppm). The same observations were made on dead larvae, pupae and adults.

Table 4. Larvicidal bio-efficacy of *Aristolochia albida* against *Anopheles gambiae*.

Extracts	Conc. (ppm)	Mean ± Std. Dev.	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
Methanol crude extract	-control	0.0 ± 0.0[a]	0.99	588.0	1141.4	0.31 ns
	500	38.3 ± 10.4[b]		(420.9 - 720.5)	(924.1 - 1666.4)	
	1000	85.0 ± 10.0[c]				
	1500	95.0 ± 5.0[c]				
	2000	100.0 ± 0.0[c]				
	Rambo	100.0 ± 0.0[c]				
	F value	131.9***				
Hexane fraction	-control	0.0 ± 0.0[a]	0.99	420.1	712.2	0.3 ns
	500	66.7 ± 12.5[b]		(151.1 - 524.1)	(575.5 - 1625.9)	
	1000	98.3 ± 2.8[c]				
	1500	100.0 ± 0.0[c]				
	2000	100.0 ± 0.0[c]				
	Rambo	100.0 ± 0.0[c]				
	F value	174.4***				
Ethyl acetate fraction	-control	0.0 ± 0.0[a]	0.97	1198.2	3039.3	0.68 ns
	500	11.7 ± 7.6[a]		(940.6 - 1538.5)	(2138.8 - 7264.4)	
	1000	43.3 ± 7.6[b]				
	1500	55.0 ± 10.0[b]				
	2000	80.0 ± 8.6[c]				
	Rambo	100.0 ± 0.0[d]				
	F value	91.6***				
Methanol fraction	-control	0.0 ± 0.0[a]	0.98	453.0	979.5	0.32 ns
	500	58.3 ± 7.6[b]		(231.9 - 594.5)	(765.6 - 1578.5)	
	1000	88.3 ± 7.6[c]				
	1500	98.3 ± 2.8[cd]				
	2000	98.3 ± 2.8[cd]				
	Rambo	100.0 ± 0.0[d]				
	F value	210.2***				

Table 5. Larvicidal bioefficacy of *Balanites aegyptiaca* against *Anopheles gambiae*.

Extracts	Conc. (ppm)	Mean ± Std. Dev.	R^2	LC_{50} (95% FL)	LC_{90} (95% FL)	χ^2
Methanol crude extract	-control	0.0 ± 0.0[a]	0.97	642.6	1338.4	1.08ns
	500	36.7 ± 7.6[b]		(460.5 - 790.1)	(1071.5 - 2016.5)	
	1000	71.7 ± 7.6[c]				
	1500	93.3 ± 11.5[d]				
	2000	100.0 ± 0.0[d]				
	Rambo	100.0 ± 0.0[d]				
	F value	119.5***				
Hexane fraction	-control	0.0 ± 0.0[a]	0.95	588.0	1683.8	2.93ns
	500	48.3 ± 5.7[b]		(311.8 - 779.9)	(1244.5 - 3522.1)	
	1000	65.0 ± 5.0[c]				
	1500	83.3 ± 5.7[d]				
	2000	100.0 ± 0.0[e]				
	Rambo	100.0 ± 0.0[e]				
	F value	285.9***				
Ethyl acetate fraction	-control	0.0 ± 0.0[a]	0.89	906.8	3408.2	1.30ns
	500	31.7 ± 5.7[b]		(551.7 - 1234.4)	(2091.3 - 9623.0)	
	1000	50.0 ± 5.0[c]				
	1500	61.7 ± 5.7[d]				
	2000	85.0 ± 10.0[e]				
	Rambo	100.0 ± 0.0[f]				
	F value	123.2***				
Methanol fraction	-ontrol	0.0 ± 0.0[a]	0.98	872.8	2550.2	0.20ns
	500	26.7 ± 5.7[b]		(595.6 - 1121.4)	(1786.6 - 6389.5)	
	1000	55.0 ± 10.0[c]				
	1500	71.7 ± 7.6[d]				
	2000	86.7 ± 2.8[e]				
	Rambo	100.0 ± 0.0[f]				
	F value	128.1***				

Table 6. Insect growth inhibitory activity of *Aristolochia albida* against *Anopheles gambiae*.

Extracts	Conc. (ppm)	% Emergence Inhibition (EI)	EI_{50} (95% FL)	EI_{90} (95% FL)	R^2	χ^2
Methanol crude extract	500	28.3 ± 11.5[a]	656.0	1249.8	0.90	43.9***
	750	63.3 ± 7.6[b]	590.1 - 713.2	1131.2 - 1432.7		
	1000	80.0 ± 5.0[c]				
	1250	86.7 ± 7.6[c]				
	1500	96.7 ± 2.8[c]				
	F value	37.9***				
Hexane fraction	500	61.7 ± 12.5[a]	482.4	562.5	0.00	13.4 ns
	750	100.0 ± 0.0[b]	405.6 - 492.8	532.8 - 962.3		
	1000	100.0 ± 0.0[b]				
	1250	100.0 ± 0.0[b]				
	1500	100.0 ± 0.0[b]				
	F value	19.109***				
Ethyl acetate fraction	500	15.0 ± 8.6[a]	1068.0	2485.8	0.80	65.0***
	750	26.7 ± 10.4[a]	956.6 - 1215.1	1955.6 - 3834.5		
	1000	46.7 ± 2.8[b]				
	1250	51.7 ± 2.8[b]				
	1500	76.7 ± 15.2[c]				
	F value	19.6***				
Methanol fraction	500	40.0 ± 5.0[a]	555.6	817.4	0.86	41.2***
	750	76.7 ± 10.4[b]	514.1 - 591.5	761.3 - 900.1		
	1000	100.0 ± 0.0[c]				
	1250	100.0 ± 0.0[c]				
	1500	100.0 ± 0.0[c]				
	F value	77.5***				

Table 7. Insect growth inhibitory activity of *Balanites aegyptiaca* against *Anopheles gambiae.*

Extracts	Conc. (ppm)	% Emergence Inhibition (EI)	EI_{50} (95% FL)	EI_{90} (95% FL)	R^2	χ^2
Methanol crude extract	500	15.0 ± 10.0[a]	921.1	1789.8	0.83	86.5***
	750	31.7 ± 5.7[a]	824.7 - 1023.3	1510.3 - 2383.8		
	1000	53.3 ± 10.4[b]				
	1250	68.3 ± 7.6[b]				
	1500	88.3 ± 16.0[c]				
	F value	22.6***				
Hexane fraction	500	40.0 ± 5.0[a]	623.9	1972.4	0.72	52.6***
	750	58.3 ± 7.6[b]	484.1 - 727.2	1555.5 - 3126.3		
	1000	73.3 ± 2.8[bc]				
	1250	71.7 ± 5.7[bc]				
	1500	86.7 ± 14.4[c]				
	F value	14.0***				
Ethyl acetate fraction	500	21.7 ± 5.[7a]	1090.7	3142.6	0.76	60.7***
	750	26.7 ± 7.6[a]	957.4 - 1287.6	2263.9 - 6091.5		
	1000	43.3 ± 14.4[ab]				
	1250	55.0 ± 13.2[bc]				
	1500	70.0 ± 8.6[c]				
	F value	10.8**				
Methanol fraction	500	13.3 ± 2.8[a]	1129.2	2802.3	0.86	42.6***
	750	26.7 ± 2.8[a]	1025.7 - 1269.9	2212.9 - 4162.2		
	1000	46.7 ± 10.4[b]				
	1250	48.3 ± 7.6[b]				
	1500	70.0 ± 13.2[c]				
	F value	19.8***				

4. Discussion

A good pesticide is the one that fulfils the following criteria: it protects non-target organisms; it is environment friendly, biodegradable and readily available all over the world [15]. Pesticides of plant origin may be considered as suitable alternatives to synthetic insecticides as they are inexpensive, eco-friendly, relatively safe on non-target organisms, easily degradable and are readily available in many areas of the world [15] [16]. For these reasons, plant derived products have received increased attention with thousands of plant species known to have pesticidal/mosquitocidal properties [17] [18] [19].

Plant secondary metabolites play an important role in plant defensive against insect herbivores and avoid infection by microbial pathogens. They also attract pollinator insects and provide a level of protection against UV light. They are classified into three main groups: terpenoids (monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, sesquarterpenes, tetraterpenes and polyterpenes), phenolic compounds (coumarin, furano-coumarin, lignin, flavonoids, isoflavonoids and tannins) and non-protein nitrogen compounds (alkaloids, cyanogenic glucosides and non-protein amino acids) [20] [21]. These secondary metabolites were found to be responsible of pesticidal activity [22].

In the present study, there was the presence of alkaloids, flavonoids, saponins, tannins and terpenoids in *Aristolochia albida* root and *Balanites aegyptiaca* seed extracts with the exception of phenols only present in *B. aegyptiaca.* The larvicidal activity of both plants extracts is comparable to that of other plant species. Keziah *et al.* [23] reported the larvicidal efficacy of *Lantana camara* and *Ocimum gratissimum* leaves extracts against fourth instar larvae of *Aedes aegypti* with the LC_{50} values of 0.37, 0.60, 0.97, 1.60 and 3.23 g/L for hexane, methanol crude, ethyl acetate, chloroform and methanol extract, respectively from *O. gratissimum* and 0.62, 0.72, 0.96, 2.20 and 3.36 g/L for ethyl acetate, hexane, methanol crude, chloroform and methanol, respectively from *L. camara*. Likewise, *Annona senegalensis* leaf hexane and chloroform fractions were more effective than other fractions on *An. gambiae* larvae with the LC_{50} values of 298.8 and 418.3 ppm, respectively while a moderate activity was also observed in hexane and chloroform fractions on *Culex quinquefasciatus* larvae with the LC_{50} values of 2087.6 and 9010.1 ppm, respectively [24]. Still in comparison to this study, Eze *et al.* [25] findings revealed that hexane, dichloromethane and acetone fractions from *Spondias mombin* were the most effective against *Ae. aegypti* with LC_{50} values of 22.5, 42.1 and 45.1 ppm, respectively. Hexane fraction registered the highest activity with LC_{50} of 92.2 ppm against *An. gambiae*. It was still hexane fraction that showed better toxicity with LC_{50} of 326.5 ppm against *Cx. quinquefasciatus*. In the same context, hexane fraction from *Callistemon rigidus* leaf has been particularly effective with LC_{50} of 56.2, 17.1 and 721.9 ppm against fourth instar larvae of *Ae. aegypti*, *An. gambiae* and *Cx. quinquefasciatus*, respectively. There was claim that this efficiency was attributed to the presence of terpenoids, steroids, lipids, fats and fixed oils which were found in the hexane fraction [26]. Similar

observations have been made where hexane fraction from *Plectranthus glandulosus* leaf showed very staggering larvicidal activity against *An. gambiae*, *Ae. aegypti* and *Cx. quinquefasciatus* fourth instar larvae with LC_{50} of 17.1, 89.1 and 610.4, respectively. Authors claimed that the activity was also attributed to the presence of steroids, terpenoids, lipids, fats and fixed oils which were present in the fraction [27]. The findings from these authors give support to those in the present study where hexane fraction was the most active and this was for both plants. The phytochemical analysis showcased the presence of alkaloids, flavonoids, saponins, tannins, terpenoids and oils in the hexane fraction of both plants plus the phenols present only in *B. aegyptiaca*. These phytochemicals might be responsible of the remarkable activity.

Insect growth regulators play a very important role in vector control. Plant derivatives are being examined for insect growth regulatory activity [6] [28] [29]. In the present study, *A. albida* root and *B. aegyptiaca* seed extracts exhibited insect growth regulatory activity against malarial vector mosquito, *An. gambiae*. Similar findings have been proved by numerous authors, indicating that plants could provide one of the biggest sources of IGRs [28].

Abutilon indicum leaf extracts have been reported to show growth inhibiting effects on *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. Their larval and pupal development was arrested resulting in decreased pupal transformation and adult emergence. Larval and pupal periods were prolonged with appearance of larval-pupal and pupal-adult intermediates, with an overall increase in the developmental period [28]. Similarly, the *Agerantum conyzoides* methanol crude extract showed demelanized *An. gambiae* larvae, abnormal *An. gambiae* larval-pupal intermediate, arrested adult emergence in *An. gambiae*, abnormal *An. arabiensis* larval-pupal intermediate and failed adult emergence in *An. arabiensis*. These different effects were believed to be partly caused by the presence of alkaloids, aglycone flavonoids, triterpenoids, tannins and coumarins [29]. In the same vein, different secondary metabolite fractions *i.e.*, alkaloid, phenolics and terpenoid caused mortality at larval and pupal stages with the LC_{50} value being the lowest for phenolic fraction. Phenolic fraction affected growth by decreasing adult life span, fertility and fecundity of *Ae. aegypti*. The reduction in growth was also accompanied by decrease in carbohydrate and lipid levels [6].

Though the modes of action of the extracts presented in this study on mosquito larvae are not known, but Pino *et al.* [22] stated that plant secondary metabolites were axonic poisons (sodium channels agonists) from *Tanacetum cinerariaefolium*, moulting inhibitors (ecdysone antagonists) from *Azadirachta indica*, mitochondrial cytotoxins from *Derris*, *Lonchocarpus* and *Tephrosia* species, neurotoxins (acetylcholine agonist) from *Nicotiana* spp., neuromuscular poisons (calcium channel agonist) from *Ryania speciosa*, insect growth regulators from *Derris indica*, suffocating agents from *Simmondsia californica* [22].

5. Conclusion

From the findings of this study, extracts from *Aristolochia albida* and *Balanites*

aegyptiaca can be considered as bold alternatives to synthetic larvicides and IGRs. They can be used for small-scale field trial in mosquito breeding sides such as containers which are known as the notorious environment for the multiplication of mosquitoes and so the still high rate of mortality by malaria. Further study needs to be carried out in order to elucidate the bioactive agents responsible of the different efficiencies, especially in hexane fraction of both plant materials. The mechanism of action of both plants extracts against *An. gambiae* larvae remains unknown and so need to be elucidated in future.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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