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Chemical Composition and Use of *Momordica charantia* L. and *Hyptis spicigera* Lam. Extracts as Mosquito Larvicides and Insect Growth Regulators against Malarial Vector, *Anopheles gambiae* Giles

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Abstract

Background: Mosquitoes are responsible of numerous vector-borne diseases and among these diseases is malaria which takes away lives of thousands of people, especially children of under five, particularly in Africa. To reduce the mortality and economic burdens of this deadly disease, synthetic insecticide has been of use to control its main vector, *Anopheles gambiae*. Due to adverse effects caused by these conventional products on non-target organisms and the environment, plants have been of first choice as they proved their effectiveness against mosquitoes and are said to be eco-friendly and relatively safer. Therefore, the aim of this study was to screen the phytochemicals and determine the larvicidal and adult emergence exhibitory activities of *Momordica charantia* and *Hyptis spicigera* leaves extracts against *Anopheles gambiae* larvae. **Methods:** The WHO standard protocol for larvicidal activity and IGRs was followed up and concentrations from 500 - 2000 ppm for larvicidal and 500 - 1500 ppm for IGRs were set up. **Results:** There was the presence of alkaloids, flavonoids, tannins, phenols, steroids, terpenoids and oils in *Momordica charantia* against alkaloids, taponins, tannins, phenols, steroids, terpenoids and oils in *Hyptis spicigera*. Methanol crude extract registered the lowest LC_{50} value of 270.6 ppm followed by ethyl acetate and hexane fractions recorded the LC_{50} of 742.1 and 756 ppm, respectively for larvicidal activity of

Momordica charantia against LC_{50} of 760 and 867.5 ppm for hexane fraction and methanol crude extract, respectively for *Hyptis spicigera*. Methanol crude extract and hexane fraction of both plants as well as the ethyl acetate fraction of *M. charantia* registered greater emergence inhibition with IE_{50} values of 590.2, 842.3 and 982 ppm for methanol crude extract, hexane and ethyl acetate fractions of *M. charantia*, respectively against 901.7 and 873.2 for methanol crude extract and hexane fraction of *H. spicigera*, respectively. **Conclusion:** The toxicity and adult emergence inhibition may be associated with constituents in both plants that interfere with the normal neuronal, respiratory and endocrine systems functions.

Keywords

Momordica charantia, *Hyptis spicigera*, *Anopheles gambiae*, Malaria, Extracts, IGRs

1. Introduction

Mosquitoes are considered as the number one enemies of mankind [1]. They are responsible of numerous vector-borne diseases namely malaria, yellow fever, dengue fever, rift valley fever, west Nile virus, Venezuelan equine encephalitis, Japanese encephalitis, Zika, la Crosse encephalitis and chikungunya [2] [3]. These diseases negatively impact the economy of many countries [2]. Malaria is the most dangerous of the arthropod-borne illnesses caused by *Anopheles* species, especially in Sub-Saharan countries [4]. As deadly as it is, malaria affected an estimated 241 million people worldwide with 228 million in Sub-African region, accounting for about 95% of cases. The estimated number of people who were killed by the disease was 602,000 in the world, with the desperate and chocking percentage of 96% in Africa (80% of all deaths in this region are among children of less than 5 years) [4]. The advent of COVID 19 has worsened the situation due to the fact that efforts in the health services were mostly oriented towards the COVID 19, retorting activities in detriments to malaria prevention and treatment [4]. In addition to disruptions in malaria diagnosis and treatment, the fear to catch COVID 19 by population in hospital reduced significantly their visit in case of suspected malaria cases during COVID 19 pandemic, increasing auto medication [4] [5].

In Cameroon malaria is highly endemic; meaning the entire population of 27 million people is exposed to the disease on a regular basis. Every year, around 6 million malaria cases with 4000 deaths are registered in health facilities, most of which occur in children below the age of five [6]. However, not all cases and deaths are recorded, and WHO estimates that about 11,000 people die from malaria in Cameroon every year. Around 30% of all out-patient visits to health care facilities are for malaria, making it a disease of importance in the country [6].

The current methods for treating malaria rely on the use of artemether, amodiaquine, artemisinin, artesunate, clindamycline, chloroquine, dihydroartemisi-

nin, 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 [4] [7]. The best option to combat malaria and other mosquito-borne diseases is to target their vectors. Efforts have been made in vector control through the use of insecticide-treated nets, indoor residual spraying, larvivorous fish, topical repellents, insecticide-treated clothing, airborne repellent, space spraying, house screening, larviciding and insect growth regulators [7]. Larviciding and IGRs are the options of first choice because the immature stages of mosquitoes are easily managed and controlled [8]. The larvae breed in a series of water-holding containers which included disposed plastic basins, car tyres, plastic containers, buckets, earthenware pots, plastic barrels, metal drums, jerry cans and poly tank [3]. Synthetic mosquito larvicides like pyrethroids, organochlorides, organophosphates and carbamates or insect growth regulators such as chitin synthesis inhibitors and juvenoids proved their undoubted effectiveness against different mosquito species [8]. However, their repetitive uses have created accumulation of toxic waste and poisoning of users, water, air and soil pollution and resistance to the control methods used [9]. These consequences have contributed to find environmentally friendly alternatives and plants, apart from their ethno-pharmacological and ethno-botanical use, have also proved their efficacy against mosquito species [10]. The phytochemicals provide better candidates for new classes of insecticides because they consist of variable components with diverse mechanisms of action that diminish the odds of development of resistance in the mosquito to the phytochemicals, generally have minimal acute toxicity to vertebrates and are environmentally safe, available and easily biodegradable [10]. They have been used as mosquito ovipositional deterrents, ovicides, larvicides, pupicides, antifeedants, insect growth regulators, repellents, attractant and adulticides [1] [10] [11] [12] [13]. They also affect insect physiology in many different ways and through various receptors which reduce the chances of developing resistance against them [14].

Momordica charantia L. (Family: Cucurbitaceae) commonly known as bitter melon is a herbaceous plant that grows around 5 m and bears simple/alternate leaves of 4 - 12 cm with 3 - 7 deeply separate lobes. It is used as a vegetable in many countries including Cameroon. In ethno-pharmacology, the fruits and leaves are useful in malaria, vermifuge, wound healing, jaundice, piles, leprosy, ulcer, diabetes mellitus, inflammation and it is found to have anti-oxidant properties. It is rich in various bioactive components such as alkaloids, minerals, steroidal saponins, vitamins, polypeptide and aromatic volatile oil [15] [16]. In India, crude fruit extract of *M. charantia* showed its mosquito larvicidal activity against three mosquito species *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* [17].

Hyptis spicigera Lam. (Family: Lamiaceae) is an erect, aromatic, annual herb

growing up to 1 metre tall [18]. The plant is widely distributed in tropical and warm temperate region [19]. It is used to treat malaria, wounds, bronchial troubles, skin diseases, diarrhea, headache, fever and cholera [18] [19]. It is known for its insecticidal and repellent activities against insects such as mosquitoes, weevils, beetles and termites [18] [19].

The present study was to report the chemical composition, the larvicidal and adult emergence inhibition activities of *Momordica charantia* and *Hyptis spicigera* leaves solvents extracts against malarial vector, *An. gambiae*.

2. Materials and Methods

2.1. Collection of Plant Materials

The *Momordica charantia* and *Hyptis spicigera* fresh leaves were collected in August 2020 early in the morning (7:00 - 9:00 GMT) from Doukoula, Mayo-Danay Division, Far-North Region, Cameroon (latitude 9˚47'381"N˚, longitude 15˚31'830"E˚, altitude of 325 masl), thoroughly washed with distilled water. They were then kept in room temperature until they got dried. The well dried leaves were ground in powdered form using mortar and pestle until the powder passed through a 0.4 mm mesh sieve. They were identified for confirmation by Dr Gilbert Haïwa, a botanist from the University of Maroua. The ground plant materials were finally stored in opaque containers inside a refrigerator at −4˚C until needed.

2.2. Extraction and Fractionation Processes of Plant Materials

The method adopted by Danga *et al.* [1] 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. Part of the methanol crude extract was first mixed with silica gel (60 - 200 mesh size) and macerated in hexane to obtain hexane fraction and marc 1. Marc 1 was dried in the laboratory and then soaked in ethyl acetate; ethyl acetate fraction and marc 2 were also separately recovered. Marc 2 after drying was soaked in methanol to collect the methanol fraction. 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 Screening of *Momordica charantia* and *Hyptis spicigera* Leaves

All extracts and fractions of both plants were used for the phytochemical

screening in order to identify the various classes of active chemical constituents following the standard prescribed methods [20] [21].

2.4. Source of *An. gambiae* Larvae

Larvae of *An. gambiae* were collected from stagnant water in a blocked gutter at Barmary quarter, Garoua 1 Sub-Division, Vina Division, North Region of Cameroon (9˚97'091"N˚, 13˚33'617"E˚, 220 masl). Larvae were kept in plastic trays containing well water in the laboratory. Larvae were fed with a diet containing crayfish and biscuit in a ratio of 3:1, respectively.

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

The determination of the toxicity of *M. charantia* and *H. spicigera* extracts against *An. gambiae* larvae was based on the WHO standard prescribed procedure [4]. Stock solutions for each extract were made by weighing 1g of each extract. Tween 80 (Polyoxyethylene sorbitan monooleate) used as emulsifier to ease the dissolution of the extracts in water. The stock solutions were made up of diluted extract and well water adjusting the aliquot to 100 ml for each extract in glass beaker.

From the stock solutions, serial dilutions were set up, making four concentrations ranging from 500 to 2000 ppm. As positive control, 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 well 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 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 24˚ - 37˚C and 22% ± 2% and photoperiod of 12L:12D. Three replicates were made for each extract and concentration and food was not provided.

For insect growth regulating (IGR) activity, the same procedure as for larvicidal activity was used with some exceptions. Five 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 terminated. Mortality or survival was counted every other day until the complete emergence of adults. The test containers were held at 22˚C - 37˚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 was ended when all the larvae or pupae in the controls were dead or

emerged as adults.

2.6. Statistical Analysis

Abbott's formula [22] was applied wherever mortality in negative control reached 5%. 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 [23] 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. Abbott's formula [22] was used wherever the percentage was between 80% and 95%. 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 [23] to determine IE_{50} and IE_{90} values using SPSS 17.0 software.

3. Results

At the end of the extraction processes, methanol crude extract, n-hexane, ethyl acetate and methanol fractions yielded 5.7%, 33.9%, 20.8% and 38.1%, respectively for *Momordica charantia* against 4.1%, 11.2%, 48.1% and 30.1%, respectively for *Hyptis spicigera* (**Table 1**).

The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, steroids, terpenoids and oils in *Momordica charantia* leaf (**Table 2**) against the presence of alkaloids, taponins, tannins, phenols, steroids, terpenoids and oils in *Hyptis spicigera* leaf (**Table 3**).

The larvicidal activity of *M. charantia* leaf extracts against *An. gambiae* 4th instar larvae showed that in general, the efficiency increased with the increase of concentrations. All extracts were effective with the methanol crude extract achieving 100% mortality at the highest concentration and 73.3% at the lowest concentration, registering the lowest LC_{50} value of 270.6 ppm. Ethyl acetate and hexane fractions recorded the LC_{50} values of 742.1 and 756 ppm, respectively. Methanol fraction was the least active with the highest LC_{50} of 1167.3 ppm (**Table 4**).

Table 5 shows the results of susceptibility of 4th instar larvae of *An. gambiae* to the *H. spicigera* leaf extracts; concentrations of 500 to 2000 ppm were evaluated, showing at 24 h post treatment lethal values of LC_{50} of 760 and 867.5

ppm for hexane fraction and methanol crude extract, respectively. Ethyl acetate and methanol were less active (Table 5).

M. charantia and *H. spicigera* leaves extracts caused *An. gambiae* larval growth disruption with mosquitoes dying at the larval stage or before completing moulting. Methanol crude extract and hexane fraction of both plants as well as the ethyl acetate fraction of *M. charantia* registered greater emergence inhibition with IE_{50} values of 590.2, 842.3 and 982 ppm for methanol crude extract, hexane and ethyl acetate fractions of *M. charantia*, respectively (Table 6) against 901.7 and 873.2 ppm for methanol crude extract and hexane fraction of *H. spicigera*, respectively (Table 7). All larvae in beakers treated with the conventional Permethrin were found dead.

Table 1. Extraction yield of *Momordica charantia* and *Hyptis spicigera* leaves.

Extract/fractions	Yields in %	
	Momordica charantia	*Hyptis spicigera*
Methanol crude extract	5.7	4.1
n-hexane fraction	33.9	11.2
Ethyl acetate fraction	20.8	48.1
Methanol fraction	38.1	30.1

Table 2. Phytochemical composition of *Momordica charantia* leaf.

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

+ (Presence), − (Absence).

Table 3. Phytochemical composition of *Hyptis spicigera* leaf.

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

+ (Presence); − (Absence).

Table 4. Larvicidal activity of *Momordica charantia* leaf extracts against 4th instar larvae of *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]	0.88	270.6	1408.8	2.57 ns
	500	73.3 ± 10.4[b]		(0.1 - 526.3)	(901.1 - 23279.1)	
	1000	78.3 ± 7.6[b]				
	1500	86.7 ± 7.6[bc]				
	2000	100.0 ± 0.0[c]				
	Rambo	100.0 ± 0.0[c]				
	F value	112.0***				
Hexane fraction	-control	0.0 ± 0.0[a]	0.93	756.0	2016.3	1.19 ns
	500	31.7 ± 7.6[b]		(505.5 - 959.5)	(1494.3 - 4015.6)	
	1000	63.3 ± 7.6[c]				
	1500	75.0 ± 8.6[c]				
	2000	95.0 ± 8.6[d]				
	Rambo	100.0 ± 0.0[d]				
	F value	100.7***				
Ethyl acetate fraction	-control	0.0 ± 0.0[a]	0.83	742.1	2397.4	2.64 ns
	500	26.7 ± 5.7[b]		(426.8 - 979.6)	(1661.1 - 6622.5)	
	1000	76.7 ± 10.4[c]				
	1500	78.3 ± 7.6[c]				
	2000	80.0 ± 10.0[c]				
	Rambo	100.0 ± 0.0[d]				
	F value	87.9***				
Methanol fraction	-control	0.0 ± 0.0[a]	0.96	1167.3	2549.3	1.15 ns
	500	11.7 ± 7.6[a]		(945.8 - 1432.7)	(1932.3 - 4614.7)	
	1000	33.3 ± 7.6[b]				
	1500	63.3 ± 15.2[c]				
	2000	86.7 ± 12.5[d]				
	Rambo	100.0 ± 0.0[d]				
	F value	58.5***				

Table 5. Larvicidal activity of *Hyptis spicigera* leaf extracts against 4th instar larvae of *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]	0.91	867.5	3020.4	1.26 ns
	500	33.3 ± 5.7[b]		(537.7 - 1156.6)	(1947.6 - 11997.5)	
	1000	48.3 ± 5.7[c]				
	1500	68.3 ± 7.6[d]				
	2000	86.7 ± 5.7[e]				
	Rambo	100.0 ± 0.0[f]				
	F value	153.1***				
Hexane raction	-control	0.0 ± 0.0[a]	0.99	760.0	1573.4	1.57 ns
	500	26.7 ± 7.6[b]		(578.4 - 920.5)	(1260.6 - 2364.1)	
	1000	633 ± 11.5[c]				
	1500	85.0 ± 10.0[d]				
	2000	100.0 ± 0.0[d]				
	Rambo	100.0 ± 0.0[d]				
	F value	105.0***				
Ethyl acetate fraction	-control	0.0 ± 0.0[a]	0.98	1656.7	8240.7	0.10 ns
	500	18.3 ± 5.7[b]		(1155.2 - 5168.2)	(3465.8 - 9393.3)	
	1000	31.7 ± 7.6[c]				
	1500	48.3 ± 2.8[d]				
	2000	56.7 ± 7.6[d]				
	Rambo	100.0 ± 0.0[e]				
	F value	137.5***				
Methanol fraction	-control	0.0 ± 0.0[a]	0.99	1177.8	4739.6	0.07 ns
	500	21.7 ± 7.6[b]		(807.4 - 1791.7)	(2600.5 - 52361.1)	
	1000	45.0 ± 8.6[cd]				
	1500	56.7 ± 10.4[cd]				
	2000	70.0 ± 10.0[d]				
	Rambo	100.0 ± 0.0[e]				
	F value	66.0***				

Table 6. Insect growth inhibition activity of *Momordica charantia* leaf extracts against *Anopheles gambiae* larvae.

Extracts	Conc. (ppm)	% Emergence Inhibition (EI)	EI_{50} (95% FL)	EI_{90} =(95% FL)	R^2	χ^2
Methanol crude extract	500	46.7 ± 5.7[a]	590.2	1763.4	0.62	111.7***
	750	58.3 ± 12.5[a]	368.4 - 729.1	1330.4 - 3700.9		
	1000	68.3 ± 12.5[ab]				
	1250	76.6 ± 18.9[ab]				
	1500	93.3 ± 7.6[b]				
	F value	6.2**				
Hexane fraction	500	16.7 ± 2.8[a]	842.3	1756.9	0.93	48.1***
	750	46.7 ± 12.5[b]	766.2 - 916.2	1523.5 - 2177.6		
	1000	60.0 ± 5.0[bc]				
	1250	71.7 ± 2.8[cd]				
	1500	86.7 ± 12.5[d]				
	F value	29.7***				
Ethyl acetate fraction	500	13.3 ± 2.8[a]	982.0	2050.0	0.92	32.1**
	750	26.7 ± 7.6[b]	916.5 - 1053.4	1789.1 - 2487.425		
	1000	58.3 ± 5.7[c]				
	1250	61.7 ± 7.6[c]				
	1500	78.3 ± 5.7[d]				
	F value	56.1***				
Methanol fraction	500	5.0 ± 5.0[a]	1237.2	2545.0	0.88	46.3***
	750	20.0 ± 5.0[a]	1135.3 - 1381.0	2093.9 - 3490.4		
	1000	36.7 ± 7.6[b]				
	1250	45.0 ± 5.0[b]				
	1500	66.7 ± 15.2[c]				
	F value	22.8***				

Table 7. Insect growth inhibition activity of *Hyptis spicigera* leaf extracts against *Anopheles gambiae* larvae.

Extracts	Conc. (ppm)	% Emergence Inhibition (EI)	EI_{50} (95% FL)	EI_{90} (95% FL)	R^2	χ^2
Methanol crude extract	500	18.3 ± 11.5[a]	901.7	2162.0	0.68	125.1***
	750	48.3 ± 7.6[b]	750.4 - 1064.1	1628.9 - 4169.5		
	1000	48.3 ± 10.4[b]				
	1250	58.3 ± 16.1[b]				
	1500	86.7 ± 14.4[c]				
	F value	11.7**				
Hexane fraction	500	11.7 ± 12.5[a]	873.2	1665.8	0.73	136.***
	750	36.7 ± 16.1[a]	751.5 - 992.8	1382.0 - 2371.0		
	1000	70.0 ± 10.0[b]				
	1250	73.3 ± 15.2[b]				
	1500	83.3 ± 15.2[b]				
	F value	13.6***				
Ethyl acetate fraction	500	5.0 ± 5.0[a]	1239.8	2440.3	0.90	37.1***
	750	16.7 ± 2.8[a]	1151.8 - 1357.1	2070.5 - 3128.4		
	1000	33.3 ± 12.5[b]				
	1250	48.3 ± 5.7[c]				
	1500	66.7 ± 7.6[d]				
	F value	31.9***				
Methanol fraction	500	1.7 ± 2.8a	1301.3	2341.2	0.91	32.7**
	750	13.3 ± 2.8a	1220.7 - 1408.4	2035.5 - 2875.8		
	1000	26.7 ± 5.7b				
	1250	43.3 ± 12.5c				
	1500	65.0 ± 5.0[d]				
	F value	40.1***				

4. Discussion

Plant secondary metabolites 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) [24] [25]. These secondary metabolites were found to be responsible of pesticidal activity [26]. Shaalan *et al.* [10] stated that: "Phytochemicals extracted from many plant species show growth inhibiting effects on the various developmental stages of different mosquito species. A range of pre-emergent effects can occur; such as prolongation of instar and pupae durations, inhibition of larval and pupal molting, morphological abnormalities and mortality especially during molting and melanization processes". In the same vein, Mulla [27] discovered that the regulatory effect on insect growth is attributed to compounds that mimic juvenile hormone in arthropods, delaying or prolonging their development or causing malformations that lead to death of the insect. In the present study, methanol crude extract and hexane fraction of *M. charantia* and *H. spicigera* leaves extracts exhibited encouraging larvicidal and adult emergence inhibitory effects on *An. gambiae*. The same achievements have been made by several authors. In previous studies, *H. spicigera* demonstrated its mosquitocidal effects on *Ae. aegypti* larvae and adults, producing 81.3% and 100% mortality for the larvae and adult, respectively [28]. The essential oil of the same plant repelled *An. gambiae* and *Cx. quinquefasciatus* adult and this repellency was attributed to the presence of alkaloids, flavonoids, saponins, steroids, tannins, terpenoids, cardiac glycoside and phlobatanins [19]. Volatile oils from *H. spicigera* showed larvicidal activity against mosquito larvae: *An. gambiae* and *Cx. quinquefasciatus* recording LC_{50} of 44.4 and 53.8 µg/mL, respectively [18]. Crude fruit extract of *M. charantia* registered 0.5, 1.3 and 1.5 ppm of LC_{50} against IV instar *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* larvae, respectively [17]. It has been demonstrated that the effect of a phytochemical depends on mosquito species, plant species, plant parts, solvents used in extraction and fractions of the same solvent [10]. In this line, methanol crude extract, hexane, chloroform, ethyl-acetate and methanol fractions from *Annona senegalensis* realized ovicidal, larvicidal and pupicidal activity on *An. gambiae* and *Cx. quinquefasciatus*; the biological activity of the plant extract was attributed to the presence of alkaloids, flavonoids, saponins, tannins, phenolic compounds, steroids, terpenoids, oil and fats [29]. The effect of *Annona senegalensis* and *Boswellia dalzielii* leaf fractions on *Ae. aegypti* showed that n-hexane and chloroform fractions of *A. senegalensis* were more effective than others with CL_{50} values of 379.3 and 595.2 mg/L, respectively and 537.1 and 585.5 mg/L for n-hexane and chloroform, respectively for *B. dalzielli*; the activity was due to the presence of alkaloids, steroids, phenolic compounds, terpenoids, fats and oils in both plants [30]. *Thevetia nerviifolia*, *Plumeria acuifolia* and *Lantana camara* ethanol extracts showed their IGR activity against *Ae.*

aegypti with IC_{50} values of 8.5, 108.7 and 317.4 ppm, respectively [31]. In addition, n-hexane, dichloromethane and methanol extracts from *Kotschya ugue-nensis* were found to possess larvicidal and adult emergence inhibitory activity against the mosquito *An. gambiae* with growth disruption by forming elongated guts and resulting in eventual death [32]. Devi *et al.* [33] claimed that different secondary metabolite fractions *i.e.*, alkaloid, phenolics and terpenoids from *Zi-ziphus jujuba* caused mortality at larval and pupal stages and affected growth by decreasing adult life span, fertility and fecundity of the mosquito *Ae. aegypti*. The reduction in growth was also accompanied by decrease in carbohydrate and lipid levels. Moreover, extracts from *Pseudocalymma alliaceum* leaves exerted larvicidal and mosquito growth inhibitory activities on *Cx. quinquefasciatus* [34].

5. Conclusion

In conclusion, it is in further search for the insecticides of plant origin that the present study was undertaken. *M. charantia* and *H. spicigera* leaves extracts indicated that they may be suitable sources of malaria vector control, especially *An. gambiae* at immature stages. It is recommended that further studies be carried out in order to illustrate the harmless effects of these extracts on non-target organisms and identify the lead compounds exhibiting the larvicidal and adult emergence exhibitory activities illustrated in the present work.

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Conflicts of Interest

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

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