J Environ Sci Public Health 2022;6 (1):031-045 *DOI: 10.26502/jesph.96120153*

Research Article

Larvicidal Activity of *Momordica foetida* (Cucurbitaceae), *Gnidia glauca* (Thymelaeaceae) and *Vepris soyauxii* (Rutaceae) Extracts on *Anopheles gambiae* Mosquitoes and their Acute Toxicity on Rats

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Received: 18 May 2021; **Accepted:** 24 May 2021; **Published:** 17 January 2022

Citation: Metoh Theresia Njuabe, Chi Tchampo Fru, Soh Desire, Herman Parfait Awono- Ambene. Larvicidal Activity of *Momordica foetida* (Cucurbitaceae), *Gnidia glauca* (Thymelaeaceae) and *Vepris soyauxii* (Rutaceae) Extracts on *Anopheles gambiae* Mosquitoes and their Acute Toxicity on Rats. Journal of Environmental Science and Public Health 6 (2022): 031-045.

Abstract

Background: The need to apply alternative strategies to control African malaria vectors such as *Anopheles gambiae s.l.* is extremely relevant. This study aimed at determining the larvicidal activity of 03 plants against *Anopheles gambiae s.l* and their acute oral toxicity.

Methods: Bioassays were carried out with third-instar larvae of *Anopheles gambiae* and *Anopheles coluzzii* mosquitoes adapted to laboratory to check for LC50 and LC90 and mortality rates, 24 and 48 hours post-exposure to aqueous and methanol extracts from *Momordica foetida*, *Gnidia glauca* and *Vepris soyauxii* plants. The testing concentrations ranged from 100 to 450 ppm. The toxicity of their methanol extracts was also evaluated using Wistar rats.

Results: Methanol extracts showed high lethal activity against *Anopheles gambiae* and *Anopheles coluzzii* larvae as compared to aqueous extracts, with variations by plant and Anopheles species as well as duration of exposure. LC50 and LC90 values recorded upon 24h exposure to *Vepris soyauxii* methanol-based extracts were 203.92 ppm and 241.46 ppm in *Anopheles gambiae* and 215.01 ppm and 270.87 ppm in *Anopheles coluzzii*, respectively. These respective LC50 and LC90 values increased with *Gnidia glauca* and *Momordica foetida* extracts. As for the toxicity, the highest concentrations of 750 ppm did not show any symptoms of toxicities and death on Wistar rats.

Conclusion: *V. soyauxii* and other study plant extracts showed promising biological responses against malaria vector species, *Anopheles gambiae* and *Anopheles coluzzii*, and may serve as potential alternative and eco-friendly tool for larval control in African endemic countries.

Keywords: Larvicidal activity; Acute toxicity; *Momordica foetida*; *Gnidia glauca*; *Vepris soyauxi*

Abbreviations

OCEAC: Organisation de Coordination pour la lutte Contre les Endémies en Afrique Centrale; OCED: Organization for Economic Cooperation and Development; LSM: Larval Source Management LC: Lethal Concentration; LD: Lethal Dose

1. Background

Malaria continue to be a major public health concern in endemic countries despite the implementation of effective antimalarial drugs for malaria management [1] and prevention. Due to the lack of an effective vaccine and the rapid spread of parasite resistance to antimalarial drugs reported, vector control is currently the main preventive strategy for malaria disease [2], because he is useful to reduce malaria transmission and to prevent the spread of resistant strains [3]. The current vector control strategy mostly relies on long-lasting insecticides treated bed nets (LLINs) and indoor residual spraying (IRS) and these two main control methods target many vectors such as *An. gambiae* sensu lato which is one of the main complex of malaria vectors in Africa especially Cameroon [4]. However, they target only the adult stage and individual endophilic and endophagous behaviours. In addition, the emergence and spread of insecticide resistance as well as behavioural changes threaten

current malaria vector control programs [5]. Also, the increasing levels of resistance to commonly used insecticides have invariably led to multiple treatments and excessive doses, posing serious threats to both the environment and human health. Considering such trends around the globe, the urgent need for the development of selective mosquito control alternatives that can serve as an alternative and complementary resistance management strategy should be addressed adequately and promptly [6]. Thus, larval source management with aim to reduce malaria by targeting immature mosquitoes, has received a renewed interest to kill or inhibit the development of mosquito larvae, by the use of chemicals, biological larvicides among others [7]. Despite the numerous measures put in place to eliminate mosquito larvae such as the application of organophosphates and insect growth regulators [8], innovative and eco-friendly solutions are needed to be promote in poor socioeconomic condition [1, 9]. Hence control of disease vector products can be done using biological materials such as plant extracts which have a potential source of bioactive compounds that are relatively safe with little site effects on the environment and human health [10]. Plants are rich sources of alternative synthetic compounds for the control of mosquito larvae. They have a wide range of bioactive phytochemicals that are selective, biodegradable, and have minor or no adverse effects on non-target organisms and the environment, making them appropriate for use in integrated pest management programs [11]. In the present study we investigate how to establish concentrations of larvicides obtained from the aqueous, and methanol extracts of the plants *Momordica foetida* (cucurbitaceae), *Gnidia glauca* (thymelaeaceae) and *Vepris soyauxii* (rutaceae) extracts ensuring its proper function and it demonstrated that there are insignificant side effects on the environment.

2. Material and Methods

2.1 Plant material collection and extraction

The plants were selected based on the literature review of phytochemicals screening, family activity and ethnobotanical information. The whole plant except the fruits of *M. foetida* was collected in Nkolbisson Yaoundé, Central Region of Cameroon in June 2019 and identified at the National Herbarium of Cameroon Yaoundé by comparison with a voucher specimen number No 33420 HNC. Fresh leaves of *G. glauca*, and stem barks of *V. soyauxii* were collected respectively on the 28th of July 2018 in Small Babanki, Tubah Subdivision, Mezam Division in the Northwest region of Cameroon and identified at the National Herbarium by comparison with voucher specimens' numbers No 40139/HNC and 38959/HNC respectively. The plant parts were cleaned with distilled water, chopped into small pieces, air-dried under shade, ground into fine powder at the Laboratory of Medicinal chemistry and Pharmacognosy of the University of Yaoundé I. The powdered samples of *M. foetida*, *G. glauca* and *V. soyauxii* were separately stored in a clean air tight polythene paper bag. Each plant powder was extracted separately using water and methanol. Water extraction was done by macerating 300 g of each dry plant powder in 2 L of distilled respectively for 72 hr at room temperature. Filtration was then done using Whatman No.1 filter paper and the filtrate concentrated using Virtis Bench Top 3® Model freeze

drier (The Virtis Company, New York), at the Institute of Medical Research and Medicinal Plant (IMPM) laboratory. The extract thus obtained was stored +4oC and was used for larvicidal bioassay. Methanol extraction was performed with 300 g of the powder of each plant through a maceration process with 2.5 L of methanol for 72 hrs at room temperature.

The extracts were filtered and concentrated with the rotary evaporator to obtain crude extracts. The resultant viscous substance was placed in amber-coloured bottles and stored in a refrigerator at +4°C and was eventually used for larvicidal laboratory tests.

2.2 Origin of *Anopheles gambiae* strains

Two strains of *An. gambiae* s.l. were used for biological tests and known as sensitive to all insecticides: *An. coluzzii* strain collected in 2005 in temporary breeding sites of the Ngousso area (Yaoundé), and *An. gambiae* ss strain, collected in 2013 in Kisumu (Kenya near Lake Victoria). Both strains were domesticated in the insectarium of the Organization for Coordination of the fight against Endemic Diseases in Central Africa (OCEAC). The two strains of *An. Gambiae* were separated for breeding in two different insectariums, free of any insecticide, following the Desfontaines et al. method, 1991 [12].

2.3 Bioassay

This was carried out following the WHO protocol [13, 14]. Twenty-five (25) larvae from each strain of *An. gambiae* were taken from their breeding areas and put in 5 cm diameter bowls, each containing 99.9 mL of spring water. Preliminary experiments were done in order to select a range of concentrations for the actual tests. Initially, the mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the materials under test [15]. After determining the mortality of larvae in this wide range of concentrations, a narrower range (of 4–5 concentrations, yielding between 10% and 95% mortality in 24 h or 48 h) was used to determine LC50 and LC90 values. Batches of 25 third instar larvae were transferred by means of droppers to small disposable test cups, each containing 100 mL of water. Small, unhealthy or damaged larvae were removed and replaced. For this purpose, stock solutions of crude extract of each sample were prepared by diluting 10 ml of extract in 10 ml of 70% ethanol. After obtaining this stock solution diluted to ½, many other dilutions were prepared from the stock solution, with varying volumes of 70% ethanol.

The following concentrations 100ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm, 400ppm and 450ppm were obtained. Four replicates were set up for each concentration and an equal number of controls were set up simultaneously with tap water, to which 1 mL ethanol was added.

The test containers were held at 25–28°C for a photoperiod of 12h light followed by 12 h dark (12L: 12D). After 24h exposure, larval mortality was recorded, same after 48h. Dead larvae were those that couldn't be induced to move when they were probed with a needle in the siphon or the cervical region. Moribund larvae were those incapables of rising to

the surface or not showing the characteristic diving reaction when the water is disturbed.

2.4 Acute toxicity test

Healthy albino rats randomly separated into four groups of 10 animals (5 males, 5 females) each were used for the oral acute toxicity test: Group 1 received distilled water and serve as control, Group 2 received methanol extract of *Momordica foetida*, Group 3 received methanol extract of *Gnidia glauca*, and Group 4 received methanol extract of *Vepris soyauxii*. The 03 test groups (2, 3 and 4) were then divided into subgroups as follows by sex of rats and doses of extract used (Table 1). All doses administered to the rats were equivalent to concentration of 450 ppm and 750 ppm (for the second set in each group) respectively yielding 100% mortality in Bioassay. The animals were observed for clinical signs, morbidity and mortality at regular interval of 4 hours on the first day and thereafter daily for 14 days. Animals that survived the test period were sacrificed by chloroform anaesthesia. Blood samples were obtained by cardiac puncture and collected in plain test tubes. The serum samples were obtained by centrifuging the blood samples at 3000 rpm for 10 minutes, and stored at -20°C until used. The activities of marker enzymes serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP) were assayed using Dialab ready to use kits.

	Group 1 (control)	**Group 2**		**Group 3**		**Group 4**	
Composition	5 males, 5 females	5 males, 5 females		5 males, 5 females		5 males, 5 females	
Test solution	Distilled water	*Momordica foetida* extract		*Gnidia glauca* extract		*Vepris soyauxii* extract	
Sub-group for toxicity tests	5 males+ 5 females	2 males+ 2 females	3 males+ 3 females	2 males+ 2 females	3 males+ 3 females	2 males+ 2 females	3 males+ 3 females
Dose administered	-	4.50 ppm	7.50 ppm	4.50 ppm	7.50 ppm	4.50 ppm	7.50 ppm

Table 1: Design of groups of Wistar rats used for oral toxicity study.

2.5 Data analysis

LC50 and LC90 values were calculated using a log dosage–probit mortality regression line in the computer software IBM SPSS version 21. Overall mosquito larvae mortality was adjusted according to Abbott's formula [16] (Abbott, 1925) when mortality in the control population was between 5% and 20%. As for the data obtained on acute toxicity they were presented as mean ± standard error of mean (SEM) of three replicate determinations. The concentrations given to the rats were latter converted to doses in mg/Kg using this formular Vol = Weight of animal /1000g x Dose.

3. Results

3.1 Phytochemical characterization of plant crude extracts

The preliminary qualitative phytochemical analysis of tested plant crude extract revealed the presence of some secondary metabolites of which some may be active ingredients for larvicidal activity. *Momordica foetida* comprised high to moderate levels of triterpenoids, steroids, sugars, glycosides, saponins and low concentrations of alkaloids, flavonoids and phenols. Alkaloids and triterpenoids in moderate concentrations, and trace of flavonoids, phenols and sterols were detected in *Vepris soyauxii* extracts, whereas flavonoids, saponins, tannins, terpenoids and steroids were found in trace in *Gnidia glauca*.

			Anopheles gambiae				***Anopheles coluzzii***			
Plant extracts		**Exposure (h)**	**LC 50 (ppm)**	**95% CI (LCL – UCL)**	**LC 90 (ppm)**	**95% CI (LCL – UCL)**	**LC 50 (ppm)**	**95% CI (LCL – UCL)**	**LC 90 (ppm)**	**95% CI (LCL – UCL)**
Aqueous extract	*Momordica foetida*	24	593.96	501.47 - 945.12	1668.92	1011.63 - 9358.32	505.19	428.61 - 849.99	1237.09	776.14 - 8828.63
		48	542.56	452.30 - 833.67	1672.04	981.97 - 2478.24	485.34	411.68 - 878.20	1177.23	733.97 - 16745.00
	Gnidia glauca	24	583.99	488.22- 950.71	1906.87	1083.50 - 2220.73	349.53	303.22 - 392.03	781.71	612.195 - 1408.75
		48	525.48	391.54 - 745.88	1863.76	1048.03 - 2579.93	324.59	290.65 - 352.28	558.18	482.31 - 758.46
	Vepris soyauxii	24	323.96	120.66 - 421.75	1113.21	817.66 - 3772.66	418.08	371.70 - 522.06	883.65	638.01 - 2841.11
		48	274.05	103.37 - 367.05	860.55	688.29 - 1681.18	399.44	347.68 - 485.01	843.47	613.80- 3015.69
Methanol extract	*Momordica foetida*	24	276.32	187.45 – 394.57	346.56	293.36 - 593.34	235.31	218.22 - 248.63	295.45	278.81 - 321.75
		48	161.89	128.02 - 185.54	249.11	225.47 - 273.18	227.25	208.11 - 240.37	264.06	249.48 - 289.96
	Gnidia glauca	24	212.42	185.68 - 233.32	348.01	325.17 - 376.71	238.43	201.77 - 262.62	336.74	306.81 - 391.96
		48	208.03	168.50 - 229.24	275.51	254.35 - 307.94	228.90	194.45 - 249.72	297.12	273.58 - 340.29
	Vepris soyauxii	24	203.92	115.91 - 220.51	241.46	223.36 - 412.50	215.01	205.61 - 222.99	270.87	260.49 - 284.82
		48	182.71	169.18 - 192.25	226.74	216.78 - 240.67	206.63	179.95 - 220.30	255.20	240.91 - 283.61

Legend: LC = Lethal Concentration, UCL = Upper Confidence Limit, LCL = Lower Confidence Limit

Table 2: Lethal concentrations (LC50 and LC90) of plant extracts at 24- and 48-hours post-exposure on Anopheles gambiae and *Anopheles coluzzii* larvae.

3.2 Lethal concentrations (LC50 and LC90)

Table 2 showed the variation of the different lethal concentrations which lead to the death of half of a population (LC50) and that which leads to 90% death of a population (LC90) after 24hrs and 48 hrs of exposure to *Momordica foetida*, *Gnidia glauca* and *Vepris soyauxii* extracts.

Though, the concentration leading to 50% mortality (LC50) and 90% mortality (LC90) after 48 hours of exposure in all extracts were lower as compared to that after 24hrs of exposure. There was no significant variation in time of exposure on these two values.

On the contrary methanol extracts yielded high values of lethal concentrations (LC50 and LC90) as compared to aqueous extracts. In *An. gambiae*, LC50 upon exposure to methanol extracts was 276.32 ppm with *Momordica foetida*, 212.42ppm with *Gnidia glauca* and 203.92 ppm with *Vepris soyauxii*, whereas this value reached 235.31 ppm, 238.43 ppm and 215.01 ppm, respectively in *An. coluzzii*. As for LC90, the same trend followed upon exposure to *Momordica foetida* (*An. gambiae*: 346.32 ppm vs. An. coluzzii: 295.45 ppm), *Gnidia glauca*.

(*An. gambiae*: 348.01 ppm vs. *An. coluzzii*: 336.74 ppm) and *Vepris soyauxii* (*An. gambiae*: 241.46 ppm vs. *An. coluzzii*: 270.87 ppm).

3.3 Mortality rates

Larval mortality varied with the extract concentration, the solvent used and the time of exposure. In comparison with the control, all of the tested extracts showed lethal effect against *An. gambiae* and *An. coluzzii* larvae at different levels of toxicity through which a progressive mortality rate trend was observed (Table 3). At the least concentration of 250ppm, mortality of *An. gambiae* was observed to be 69% after 24hrs and 90% after 48hrs, 95% after 24hr and 96% after 48hr, and 69% after 24hr and 91% after 48hrs upon exposure the methanol extract of *M. foetida*, *G. glauca* and *V. soyauxii* respectively. The methanol extract upon exposure of 250 ppm, of *M. foetida*, *G. glauca* and *V. soyauxii*, respectively yielded mortality rates on *An. coluzzii* of 74% after 24hr and 89% after 48hr, 70% after 24hr and 80% after 48hrs and 79% after 24hrs and 88% after 48hrs respectively. In *An. gambiae* the methanol extracts highest concentration of 450 ppm, mortality rates of 93% after 24 hrs and 99% after 48 hrs, 96% after 24hrs and 97 hrs, and 94% after 24hrs and 48hrs upon exposure to *M. foetida*, *G. glauca* and *V. soyauxii* respectively. In *An. coluzzii*, mortality rates upon exposure to methanol extracts of plant at the concentration of 450 ppm where, 94% for both 24 and 48 hours with *M. foetida*, 96% after 24 hrs and 97% and 48 hrs *G. glauca* and 96% after 24 hrs and 97% after 48 hrs with *V. soyauxii*.

Plant extracts		**Exposure (h)**	***Anopheles gambiae***							***Anopheles coluzzii***					
			Control	**250**	**300**	**350**	**400**	**450**		**Control**	**250**	**300**	**350**	**400**	**450**
Aqueous extract	*Momordica foetida*	24	0	43	48	55	60	66		0	22	29	36	42	48
		48	0	51	55	59	68	76		0	26	35	40	47	53
	Gnidia glauca	24	0	22	28	30	36	43		0	33	44	56	62	70
		48	0	28	32	37	43	50		0	40	53	63	69	73
	Vepris soyauxii	24	0	17	24	28	33	40		0	27	35	41	57	64
		48	0	25	30	36	42	47		0	33	40	46	63	75
Methanol extract	*Momordica foetida*	24	0	69	83	92	93	93		0	74	89	94	94	94
		48	0	90	93	93	96	99		0	89	94	94	94	94
	Gnidia glauca	24	0	95	95	96	96	96		0	70	86	90	96	96
		48	0	96	97	97	97	97		0	80	93	96	97	97
	Vepris soyauxii	24	0	69	82	94	94	94		0	79	96	96	96	96
		48	0	91	93	94	94	94		0	88	97	97	97	97

Table 3: Mortality rates (in percentage) of *Anopheles gambiae* and *Anopheles coluzzii* following exposure to lethal plant extract concentrations (in ppm).

3.4 Acute toxicity of plant extracts

The evaluation of acute toxicity consisted of the direct observation of behavioural responses of rats following ingestion of methanol plant extracts, and the dosage of some biochemical parameters in blood of rats such as Serum Aspartate transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP). The mean weight of 40 wistar rats used was 179 ± 24 g. concerning the behavioural responses, the acute toxicity studies performed with the doses ranging from 1100 to 2261 mg/kg did not resulted in death of any animal for fourteen days of observation within the experimentation period.

Though, two animals from Group 1 displayed some restlessness. There was also the case of a rat with fur shedding upon administration of the extracts. But then it wasn't considered that significant since it was observed that majority of the animals did not display such responses (Table 4).

No.	Response	Group 1 (as control)	Group 2	Group 3	Group 4
1.	Alertness	+	+	+	+
2.	Restlessness	-	-	-	-
3.	Grooming	-	-	-	-
4.	Touch response	+	+	+	+
5.	Pain response	+	+	+	+
6.	Tremors	-	-	-	-
7.	Salivation	+	+	+	+
8.	Pupils	+	+	+	+
9.	Food intake	+	+	+	+
10.	Water intake	+	+	+	+
11.	Fur shedding	+	+	+	+
12.	Writhing	-	-	-	-
13.	Gripping	+	+	+	+
14.	Skin colour	+	+	+	+

Legend: -: absence, +: presence

Table 4: observable responses of rats to distilled water (control) and to MM (group 2), GG (group 3) and VS (group 4) extracts.

Related to biochemical parameters in blood, values of Serum Aspartate transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP) in groups of rats treated with methanol extracts were within the reference range, and did not show significant variations compared with the values of the control animals (Table 5). In addition, there was any difference between male and female animals. Hence, LD50 of the methanol extracts of the various plants could also be thought of to be larger than 22601 mg/kg.

Sex	Plant extract (doses administered in mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)
Male	G 1 (Control)	9.13	11.32	112.71
	G 2a (1117.3)	9.27	11.46	117.26
	G 2b (2039.7)	9.33	11.47	115.95
	G 3a (1164.4)	9.22	11.37	117.73
	G 3b (1909.0)	9.33	11.44	114.53
	G 4a (1119.4)	9.35	11.47	113.07
	G 4b (2032.9)	9.43	11.41	119.75
Female	G 1 (Control)	7.92	10.21	86.23
	G 2a (1530.3)	8.01	10.52	84.31
	G 2b (2220.2)	8.17	10.39	83.81
	G 3a (1422.9)	7.96	10.45	88.79
	G 3b (2227.9)	7.94	10.41	85.13
	G 4a (1427.3)	7.98	10.41	86.39
	G 4b (2260.7)	8.06	10.44	87.94
Reference values		<12	<12	73-207

Legend: G: group, a, b: subgroup

Table 5: Ranges of Serum Aspartate transaminase (AST), Alanine Transaminase (ALT), and Alkaline Phosphatase (ALP) enzymes of rats of control and test groups.

4. Discussion

Pharmacological extracts and formulation tests are essential to be performed to observe the security and effectiveness of merchandise like individual compounds, mixture of compounds, crude extracts, reaction intermediates, pesticides, finished products, pharmaceutical excipients and aids, medicines, cosmetics and other chemical ingredients. It has been recommended that healthful herbs would be in the amplest supply to spread them on larger scales to fulfil the increasing demands. Hence, such beneficial plants extracts should be evaluated for higher applications of their healthful benefits, safety and efficacy [24].

Presently, environmental safety is of paramount importance hence larvicides which are eco-friendly in nature and does not cause mortality on non-target organisms are quite acceptable. It is in this line we have designed our study using three plants with various medicinal uses by local populations in Cameroon. Phytochemicals which are relatively safe,

inexpensive and readily available in many plants are found in different parts of the world. These plants being used for traditional medicine and/or consumption can serve as mosquito larvicides leading to a reduction in the transmission malaria. The biological activity of plant extracts is generally known to be due to the presence of various bioactive phytomolecules present in the plant, including alkaloids, terpenoids, and phenolics [20].

The three plants in this study were observed to have greater larvicidal activity despite the use of polar solvents. *G. glauca*, was seen to have the lowest larvicidal activity with values of LC50 238.43 ppm and LC90 336.74 ppm as compared to the use non polar solvents on V. grandifolia chloroform extract, [17] which showed LC50 and LC90 values of 370 ppm and 703 ppm respectively against *An. gambiae*. While V. soyauxii being the plant with the highest larvicidal activity with values of LC50 215.01 and LC90 270.87 ppm is seen to have a lower larvicidal activity as compare to acetate extract of Aloe turkanensis (Asphodelaceae) which had LC50 and LC90 values of 180 and 342 ppm respectively on exposure to *An. gambiae* [18]. Furthermore, the aqueous extract of these plants was seen to have an LC50 in the ranges of 323 to 593 ppm and an LC90 in the ranges of 860 to 1800 ppm. These results were in line with the extract of Cleistanthus collinus (Euphorbiaceae) leaves having an LC50 value of 409.77 and LC90 value of 831.08 ppm when exposed to *An. gambiae* [19]. The variation in phytochemical composition could explain the differences in LC50 values, according to the extract and the part of the plant used. Likewise, in general, the higher the time of exposure the lesser the LC50. Larvicidal activity of *Gnidia glauca* plant can be attributed to the presence of active phytochemicals from methanol extract which revealed the presence of tannins, terpenoids, steroids, saponins and flavonoids. This correlates with previous reports in which *G. glauca* was observed to have larvicidal properties [21], but then in his study it was observed that its LC50 values were low as compared to this study, as it was exposed on Aedes spp rather than Anopheles spp. *Vepris soyauxii* stem barks which was used has been shown to have great larvicidal activity as it is reported to have alkaloids, flavonoids, phenols, saponins, tannins, triterpenes [22] which are the phytochemicals that cause mortality in mosquitoes [19]. *Momordica foetida* being one of a greatly used traditional plant, contain alkaloids, flavonoids and xanthine which reported to exhibit larvicidal activity [23], hence the mode of action.

Safety of the methanolic extracts of *Momordica foetida* (whole plant except fruits), *Gnidia glauca* (leaves) and *Vepris soyauxii* (stem bark) were evaluated principally by estimation of acute oral toxicity. The first signs of toxicity are usually adverse effects which include changes in body weight, and general behaviours, and thus critical for the objective assessment of the effect of a compound on test animals [25]. There were no apparent noxious signs in the animals treated. This may suggest that the extract does not provoke acute toxic response in the treated animals. Furthermore, blood chemistry parameters which could potentially ascertain damage to liver, kidney, heart and other internal organs [26] showed that animals treated with the methanolic extracts of

the plants in the study had values within the reference range. Within the study, even the highest dose of plant extract i.e., 2220.2mg/kg in *M. foetida*, 2227.9mg/kg in *G. glauca* and 2260.7mg/kg in *V. soyauxii* did not show any symptoms of toxicity and death within the animals. This implies that the Lethal Doses resulting to 50% death of the population (LD50) from these extracts are greater than their average doses of 2220.2mg/kg in *M. foetida*, 2227.9mg/kg in *G. glauca* and 2260.7mg/kg in *V. soyauxii*. This result is consistent with previous reports on *Gnidia glauca* (Fresen.) Gilg [27] methanolic extract in Albino Rats as per OECD Guideline 425 [28], on *Momordica foetida* (Cucurbitaceae) being used as an anti-malaria medicine [29] and on *Vepris soyauxii* (Rutaceae) as potential anti-cancer medicine [30]. In addition, biochemical parameters as potential indicators of damage to liver, kidney, heart and other internal organs [26], remained within the reference range values. This suggests that the three plants could be recommended as safe and offers no harm to the animals, and therefore be further investigated in search of active compounds against mosquitoes.

5. Conclusion

From our present study, we concluded that the aqueous and methanolic plant extracts of *M. foetida*, *G. glauca* and *V. soyauxii* against *Anopheles gambiae* s.l. larvae showed lethal activity, with *V. soyauxii* methanolic extract exhibiting the highest activity in laboratory in both *An. gambiae* s.s and *An. coluzzii* species. Hence, *V. soyauxii* extracts showed promising in vitro responses against larval stages of Anopheles mosquitoes. They could serve as potential alternative and eco-friendly tool for larval control in malaria endemic countries.

Acknowledgments

The authors are grateful to all study participants and technical staff of OCEAC for their participation and for providing facilities for laboratory work in the likes of Dzo Ulrich and Laurelle Djieukap. We acknowledge Motoum Tedjo Florette who supplied the rats and Joelle Tanguep who assisted in mosquito rearing.

Funding

This study was financially supported by OCEAC, Dr Parfait, Dr Soh and Dr Metoh. The funder had no role in the design of the study, collection, analysis, interpretation of data or in writing the manuscript.

Availability of Data and Materials

All data generated or analysed during the study are included within this published article.

Authors' Contributions

MTN and HPAA conceived the study, designed and supervised the experiments and data analysis, CTF performed bioassay and toxicity experimental test, data analysis and drafted the manuscript, SD harvested, extracted plant crude extract and revised the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

All the reported experiments on animals were in accordance with the OECD guidelines [28] which were laid down in the early 1980s (then repeatedly

updated) to regulate the toxicity testing of pharmaceutical substances on animals.

Conflict of Interests

The authors declare that they have no conflict of interests.

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