LARVICIDAL EFFECT OF CRUDE EXTRACTS OF *STRIGA HERMONTHICA* (Delile) AGAINST *ANOPHELES, CULEX* AND *AEDES* MOSQUITOES IN KATSINA STATE, NIGERIA

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Abstract: In this research, the effect of aqueous, methanolic and chloroform extracts of the flower, leaves and whole plant of *Striga hermonthica* against the species of *Aedes*, *Anopheles* and *Culex* mosquitoes' larvae were investigated. The plant specimen was obtained within Katsina State between June and September 2019. The plants were identified in the department of Biology, Umaru Musa Yar'adua University Katsina. Extraction was carried out using the conventional maceration technique. The phytochemical test of the plant extracts was carried out using combined standard procedure and TLC to ascertain the findings from phytochemical screening. Mosquito larvae of *Aedes, Anopheles* and *Culex* species were collected and identified from their breeding sites around the Katsina metropolis. Physicochemical assessment of the water from which the larvae was collected was also determined. The Larvicidal bioassay was done according to the laid procedure of the World Health Organization (WHO). Atomic absorption spectroscopy (AAS) was used to identify the possible mechanism of action through which the extract has larvicidal activity. The extract from the three plant parts has shown the presence of secondary metabolites. All tested extracts showed larval mortality, however, larval mortality of flower was greatest in aqueous extracts (93.35%) at 48 hours of exposure, while the lowest mortality was observed in aqueous extracts of 0.1 % with (1.67%) in flower, leaves and whole plant respectively. Percentage mortality after 12 hours of exposure at 1.0 % in leaves and whole plant showed less larval mortality compared to flower aqueous extract at high concentration. The larval mortality in chloroform extracts also showed the highest mortality (91.67%) at 1.0% with chloroform flower extract after 48 hours of exposure. The low concentration of 0.2% showed the lowest larval mortality (1.67%) after 12 hours of exposure to the flower. The larval mortality was also great with methanol extract after 48 hours of exposure on the whole plant while the lowest was observed in flower and leaves at a low concentration of 0.2% after 12 and 24 hours of exposure. It was found that all parts of *Striga hermonthica* contain Larvicidal properties that could be developed as larvicides for mosquito control. Atomic spectroscopy absorption indicates the concentration of the Na+, K+, Cu2+ and Mg+ ions present in the control and treated samples in an attempt to determine the mechanism of action viz: Na+/K+ATPase. The control group showed a decreased concentration of the K+ and consequent increase in Na+, which was reversed in the treated sample.

Keywords: Mosquito larva, Striga hermonthica, *Anopheles, Culex* And *Aedes* mosquito larvae.

INTRODUCTION

Several mosquito species belonging to genera *Anopheles*, *Culex,* and *Aedes are* vectors for the pathogens for various diseases like malaria, filariasis, Japanese encephalitis, dengue, yellow fever, chikungunya (Redwane *et al.*, 2002). Mosquito-borne diseases are still one of the major public health problems in both human and veterinary sectors affecting the socio-economic status of many Nations (Bagavon and Abdurrahman, 2011).

Insecticide resistance is now a major problem facing malaria vector control programs in most African countries with all the three main mosquito species, *A. gambiae, A. arabiensis* (of the *A. gambiae* complex) and *A. funestus*, showing resistance to one or more of the insecticide classes used in vector control (Coetzee, 2004). So, due to the dramatic increase in resistance of mosquitoes to familiar chemicals in the absence of new compounds, better alternative means of control are sought (Tripathi *et al.*, 2003).

Striga hermonthica (L), commonly known as Purple witchweed, or giant witchweed, is a hemi parasitic plant that belongs to the family *Orobanchaceae*. *S. hermonthica* is also well-known to have a devastating impact on many important food cereal crops in Africa (Koua, 2011).

Katsina town is located on the coordinates 12° 15' North and 7° to 30' East, it is one of the poorest states in north western Nigeria, with the population of 5,801,584 (NPC, 2006)

A study conducted on species of mosquitoes found in Katsina state shows the abundance of *Anopheles- gambiae* (43.3%), *Culex pipiens* (26.8%), *Anopheles arebiensis* (15.1%) and *Anopheles funestus* (13.9%) and it is reported to be more abundant during the rainy season with the high population within the metropolitan area (Bunza *et al.,* 2010). Botanicals with larvicidal potentials are now recognized as important alternative insecticides to replace synthetic ones in mosquito larvae control program due to their excellent larvicidal activities (Das and Rabha, 2007). Abdullah *et al.* (2017), reported that *Striga hermonthica* aqueous extract demonstrated larvicidal potency of about 100% mortality attained at day 3 at 1.0 and 0.5% concentration respectively Moreover, Jawale (2014), also reported that plant containing saponin have various biological activities ranging from membrane permeability, hemolytic function, and immune-stimulant and also increase mortality of insect.

MATERIALS AND METHOD

Physicochemical Properties of Water

Method of Dejenie *et al.* (2011) was used with some modification, where water samples were collected from different breeding sites of mosquitoes in katsina. The samples of water were collected in dark coloured 25-litre jerrycan which has been previously treated with 10 % HNO_3 and 1:1 HCl for 48 h. washed, rinsed with deionized water and dried overnight and Dissolved oxygen were determined using Adebote, 2010 guidelines.

Figure 1. Measurement of pH and Dissolved oxygen

The study area

This study was conducted in Katsina state, Northern Nigeria. Katsina state, which covers an area of 23,938 sq. km, is located between latitudes 11 08' N and longitudes 6 52' E and 9 20' E. The state is bonded by Niger Republic to the north, Jigawa and Kano states to the east, Kaduna state to the south and Zamfara state to the west. The state has 34 Local Government Areas.

Collection of Plant Material and mosquitoes' larvae

Fresh samples of the plant (*Striga hermonthica*) were collected within Katsina state and the plants names were authenticated in the herbarium, department of Biology, Umaru Musa Yar' adua University Katsina and voucher numbers were given as UMYU2048.

The larvae of *Anopheles, Aedes* and *Culex mosquitoes* were collected from stagnant water- ponds, discarded tires in and around the study area. The mosquito larvae were identified by an Entomologist and further confirmed using Kent and chester (2005) guiding manuals.

Extraction

Extraction was carried out using the conventional maceration technique, in the way that it could be repeated in any remote areas where the plants grow naturally the method of extraction was the same for all the plant parts. The collected samples were washed and sun-dried, after which, the sample was ground to powder. 100 g of the ground material was placed into 500 ml beakers, 200 ml of distilled water, 95% methanol and chloroform were added and mixed vigorously. The mixture was kept for 24h, filtered using a fine muslin cloth and the mixture was then concentrated under water birth till the extract was allowed to dry into semi-solid state, the extract was used for phytochemical screening.

Phytochemical investigation of the investigated plant parts

The various solvent extracts were subjected to preliminary phytochemical screening according to the procedures and methods described by Sofowora, (1993).

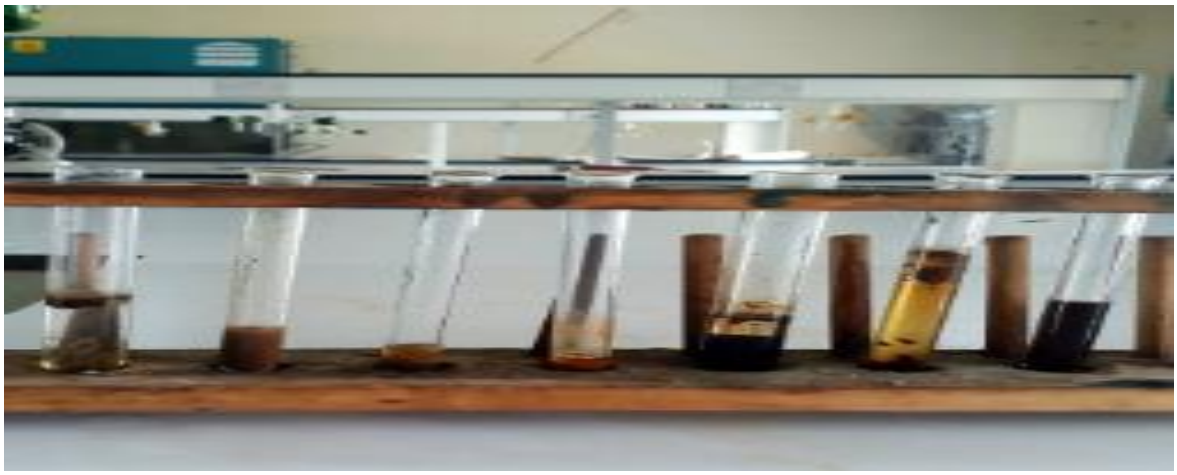

Figure 2.

Reagents

All the reagents used are of analytical grades including, distill water, chloroform, methanol. hydrochloric acid (HCL), sulphuric acid (H_2SO_4).

TLC method analysis

The TLC method reported by Hassan *et al.* (2015), was adopted and modified for this study. The method reported using methanol and ethyl acetate (1:2 v/v) as the mobile phase. However, in this study, the mobile phase composition is n-hexane: ethyl acetate: methanol (5:2:1 v/v) with the resulting Rf (Retention factor) value of around 0.56.

Dose-response bioassay

Stock solutions of each of the three solvent based extracts of the plant's parts were made with distilled water in separate labelled flasks. Dilutions of the stock solutions were done to obtain diagnostic concentrations to which the mosquito larvae were exposed. Tentatively, concentrations of 1.0, 0.5, 0.2 and 0.1%/w/v were used. The disposable cups containing the larvae were kept in netted enclosure in the laboratory and fed on with yeast tablet and glucose in the ratio of 1:10, disposable cups of (100 ml), were arranged on a clean sterilized laboratory bench and labelled "a, b, c, and d with three replications and 'e' serving as the control. The cups of "a, b. c. d, and e" contained 50 ml

of the extracts of 1.0, 0.5, 0.2 and 0.1% concentrations respectively. Twenty larvae of third instars were placed into each cup containing 50 ml of the treatment solutions and the effects of the extracts on the larvae were monitored at regular intervals of 12, 24, 36, and 48, hours after treatment. Larvae are considered dead if settled and remained motionless at the bottom of the test container with no response to light or mechanical stimulus.

Determination of the possible mechanism of action of the extract

The second and third instar larvae of *Aedes* was used in the experiment due to its higher percentage mortality with low-level concentration in Aqueous flower extract used, the possible mode of Larvicidal action of the constituents was elucidated using biochemical methods. Table.6. shows the concentration of Sodium, Potassium, Magnesium and copper in the treated and control samples. It can be seen that there was a decrease in Sodium and a corresponding increase in Potassium concentration which is indicative of the extracellular concentration of this ion (WHO-CDS, WHOPES GCDPP, 2005).

Statistical analysis

The data were analyzed statistically using a SPSS-IBM version 22 California Inc. Percentage mortality at regular intervals of 12 hours was calculated and the result were compared (differences between the means) using Analysis of variance (ANOVA) at a p-value of < 0.05 and Chi-square test at the same level of significance. The results were presented in tables.

RESULTS

Physicochemical Properties of Water

Table 1: Physicochemical parameters of water in breeding sites of mosquitoes *Aedes*, *Anopheles* and *Culex* in Katsina, Nigeria

Mosquito larvae	pH	Temperature (°C)	D.O mg/l	
Aedes	6.13-7.1	15-25	4.60-7.11	
Anopheles	8.20-9.0	27.0-33.0	4.80-5.10	
Culex	7.14-7.30	27.3-29.0	2.43-3.84	

Table 2: Preliminary phytochemical screening of some botanicals using chloroform, methanol and aqueous extract

S. hermonthica

	ALk	PHT	SPN	TN	FLV	TR	CGC
Aqueous Extract							
Flower	+	+	+	+	+	+	+
Leaves	+	+	+	+	+	+	+
Whole plant	+	+	+	+	+	+	+
Methanol extract							
Flower	+	+	+	+	+	+	+
Leaves	+	-	+	+	+	+	+
Whole plant	+	+	+	+	+	+	+

Chloroform extract							
Flower	+	+	+	+	+	+	+
Leaves	+	+	+	+	+	+	+
Whole plant	+	+	+	+	+	+	+

Key: AKL = Alkaloids; PHT = Phytosterols; SP= Saponins; PHC = Phenolic compounds; TN = Tanins; FLV = Flavonoids; TR = Terperoids; CGC = Cardiac glycosides; + = Presence; - = Absence

Table 3: Percentage mean mortality of aqueous extract of different parts of *Striga hermonthica*

	Treatment	Percentage mean Mortality				Lethal Concentration (%w/v)		
		Concentration (%w/v)						
Aqueous Extract	Time(h)	1.0	0.5	0.2	0.1	P-value	LC_{50}	LC_{90}
Flower/Ae.	12	5.00 ± 0.00^{c}	5.00 ± 0.00^{b}	3.35 ± 0.58^{c}	0.00 ± 0.00^{a}	0.001	2.319	33.711
	24	31.67 ± 1.15^{b}	13.35 ± 0.00^{a}	5.00 ± 0.00^{c}	1.67 ± 0.58^{a}	0.002	1.178	27.592
	36	35.00 ± 1.17^{b}	13.35 ± 1.15^{a}	5.00 ± 1.00^{c}	3.33 ± 0.58^{a}	0.009	1.226	62.732
	48	93.35 ± 0.58^{a}	21.67 ± 1.15^{a}	8.33 ± 0.58^{c}	8.35 ± 1.00^{a}	0.138	2.759	4.303
Leaves/An.	12	5.00 ± 0.00^{a}	3.35 ± 0.58^{a}	0.00 ± 0.00^{d}	0.00 ± 0.00^{d}	0.000	1.945	10.350
	24	10.00 ± 0.00^{b}	5.00 ± 0.00^{b}	10.00 ± 0.00^{a}	0.00±0.58a	0.000	1.492	8.183
	36	23.35 ± 0.58^{c}	13.35 ± 0.58^{c}	1.67 ± 0.58^{a}	1.67 ± 0.58^{a}	0.001	1.211	19.830
	48	60.00 ± 0.00^{d}	18.35 ± 0.58^{a}	3.33 ± 0.58^{a}	1.67±0.58	0.019	0.741	65.688
Whole plant/Cu.	12	13.35 ± 0.58^{b}	5.00 ± 0.00^{c}	1.67 ± 0.58^{a}	0.00 ± 0.00^{a}	0.000	1.294	8.875
	24	31.67 ± 1.15^{c}	11.67 ± 0.58^{c}	5.00 ± 0.00^{b}	5.00 ± 0.00^{d}	0.115	1.803	1.563
	36	33.34 ± 0.58^{c}	16.67 ± 0.58^{d}	5.00 ± 0.00^{b}	5.00 ± 0.00^{d}	0.102	0.896	6.596
	48	91.67 ± 0.58^{d}	21.67 ± 1.15^{d}	8.33 ± 0.58^{d}	6.67 ± 0.58^{d}	0.792	2.075	1.233

Values with the same superscripts (a-c) in the same column are significantly the same at $P< 0.05$ (n=3)

Table 4: Percentage mean mortality of methanol extract of different parts of *Striga hermonthica* used and exposure time

	Treatment	Percentage Mean Mortality					Lethal Concentration (%w/v)	
		Concentration (%w/v)						
Methanol Extract	**Time(h)**	**1**	**0.5**	**0.2**	**0.1**	**p-value**	**LC_{50}**	**LC_{90}**
Flower/Ae.	12	6.67±1.15^{d}	3.35±0.58^{c}	1.67±0.58^{c}	0.00±0.00^{a}	0.00	1.955	15.295
	24	15.00±0.00^{c}	11.67±0.58^{d}	1.67±0.58^{b}	0.00 ±0.00^{a}	0.00	0.959	8.002
	36	26.67±0.58^{b}	16.67±0.58b	3.35±0.58^{c}	0.00±0.00^{a}	0.00	1.038	13.449
	48	55.00±0.00^{a}	23.35±0.58^{a}	3.35±0.58^{c}	0.00±0.00^{a}	0.01	1.906	23.633
Leaves/An.	12	5.00±0.00^{d}	3.35±0.58^{d}	1.67±0.58^{a}	0.00±0.00^{a}	0.001	2.430	31.569
	24	18.35±1.15^{c}	10.00±0.00^{c}	1.67±0.58^{a}	0.00±0.00^{a}	0.000	0.876	6.107
	36	26.67±0.58^{b}	16.67±0.58^{b}	3.35±0.58^{c}	0.00±0.00^{a}	0.001	1.906	23.633
	48	60.00±0.00^{a}	21.67±0.58^{a}	3.35±0.58^{c}	0.00±0.00^{a}	0.001	0.995	17.518
Whole plant Cu.	12	8.35±0.58^{d}	3.35±0.58^{d}	3.35±0.58^{a}	0.00±0.00^{a}	0.000	1.873	2.124
	24	20.00±0.58^{c}	10.00±0.00^{c}	3.35±0.58^{a}	0.00±0.00^{a}	0.000	0.777	2.269
	36	30.00±0.00^{b}	18.35±0.58b	5.00±0.00^{a}	0.00±0.00^{a}	0.002	0.845	21.426
	48	68.35±0.58^{a}	23.55±0.58^{a}	5.00±0.00^{a}	0.00±0.00^{a}	0.002	1.915	36.371

Values with the same superscripts (a-c) in the same column are significantly the same at P< 0.05 (n=3

Table 5: Percentage mean mortality of chloroform extract of different parts of *Striga hermonthica* used and exposure time

		Percentage mean Mortality					Lethal Concentration (%w/v)	
		Concentration (%w/v)						
Chloroform Extract	Time(h)	1.0	0.5	0.2	0.1	P-value	LC_{50}	LC_{90}
Flower Ae.	12	11.67±0.58^{c}	6.67±.58^{b}	1.67±0.58^{d}	0.0±0.00^{a}	0.000	1.351	20.017
	24	16.67±0.58^{c}	11.67±0.58^{b}	3.33±0.58^{c}	0.00±0.00^{a}	0.000	0.915	7.330
	36	31.67±0.58^{b}	18.33±0.58^{a}	6.67±0.58^{b}	0.00±0.00^{a}	0.001	0.899	16.318
	48	91.67±0.58^{a}	23.33±0.58^{a}	6.67±1.15^{b}	0.00±0.00^{a}	0.000	1.879	15.360
Leaves An.	12	8.33±0.58^{d}	6.67±0.58^{c}	0.00±0.00^{b}	0.00±0.00^{a}	0.000	1.561	9.874
	24	15.00±0.00^{c}	10.00±0.00^{c}	6.67±0.58^{a}	0.00±0.00^{a}	0.000	1.046	6.450
	36	26.67±0.58	15.00±0.00^{b}	10.0±0.00^{a}	0.00±0.00^{a}	0.023	0.878	88.033
	48	61.67±0.58^{a}	26.67±0.58^{a}	11.00±0.58^{a}	0.00±0.00^{a}	0.026	1.861	79.62
Whole plant Cu	12	28.35±0.58^{c}	8.35±0.58^{b}	3.35±0.58^{a}	3.35±0.58^{a}	0.001	1.601	22.653
	24	31.67±1.15^{b}	11.67±0.58^{b}	5.00±0.00^{b}	3.35±0.58^{c}	0.115	1.803	1.563
	36	65.00±0.00^{b}	18.33±0.58^{a}	6.67±1.15^{c}	3.35±0.58^{c}	0.061	0.683	72.375
	48	90.00±0.00^{a}	18.33±0.58^{a}	6.67±1.15^{c}	3.35±0.58^{c}	0.061	0.683	72.085

Values with the same superscripts (a-c) in the same column are significantly the same at P< 0.05 (n=3)

Table 6: Atomic absorption spectroscopic analysis of the *Aedes larvae* treated with an aqueous extract of *Striga hermonthica* leaves

Sample	Metallic concentration			
	Na (ppm)	**K (ppm)**	**Mg (ppm)**	**Cu (ppm)**
Control group	0.1751±001	0.0646±0.01	0.3914±0.01	0.2687±0.00
Treated group	0.0311±0.00	0.1845±0.01	0.4199±0.03	0.1503±0.00

Discussion

The percentage yield aqueous extracts of *Striga hermonthica* flower, leaves and whole plant shows high yield in whole plant followed by flower and leaves . The Phytochemical screening of aqueous, methanol and chloroform extracts of *Striga hermonthica* flower, leaves and whole plants revealed the presence of various secondary metabolites. Alkaloids, flavonoids, tannins, Terperiods, saponins, cardiac glycosides were detected in the aqueous extracts. In the methanolic extracts of the leaves, phytosterols were absent. However, the chloroform extracts of flower, leaves and whole plants revealed the presence of all the secondary metabolites tested as displayed in Table 2 The differences in metabolites composition might be due to the polarity of the solvents and their ability to dissolve compounds having the same polarity.

This agrees with the findings of Gutierrez *et al.* (2014); Jawale (2014) and Abdullahi *et al.* (2011), who reported that *Striga hermonthica* is rich in alkaloids, flavonoids, tannin, steroids/triterpenoids saponin and cardiac glycosides and are having larvicidal and insect repellant activity.

The aqueous extracts of flower, leaves and whole plant of *Striga hermonthica* demonstrated larvicidal activity against third and fourth instar larvae of *Aedes, Anopheles and Culex* mosquitoes with 93.33 %, 60.00 %w/v and 91.67 %w/v with corresponding LC_{50} and LC_{90} of 2.759,4.303,0.741,65.688, and 2.075,1.233 respectively Table 3. The differences in the efficacy of the aqueous extracts of the flower of the studied plant at 1.0 % concentration at different periods of exposure was not significant ($P<0.05$).

The mortality could be attributed to the presence of many phytochemicals such as flavonoid, saponin terpenoids, alkaloid and sterol in aqueous extract, this finding correlates those made by Abdullah *et al.* (2011), who reported that *Striga hermonthica* aqueous extract demonstrated larvicidal potency of about 100 % mortality attained at day 3 at 1.0 and 0.5 % concentration respectively. Moreover, Jawale (2014), also reported that plant containing saponin have various biological activities ranging from membrane permeability, hemolytic function, and immune-stimulant and also increase mortality of insect.

The highest larvicidal activity of methanol extract on larvae of mosquitoes in the report referred to table 4 was noted at a concentration of 1.0 %w/v at various exposure time in which whole plant methanolic extract shows highest mortality of 68.35% against *Culex* at 48h of exposure with LC_{50} and LC_{90} of 1.915, 36.371 and the differences among the various time of exposure used is significant at ($P< 0.05$). This could be due to the toxicity developed upon accumulation of the extract with time. It also revealed that all tested concentrations of the extracts resulted in mortality except the control group. Morever, Jawale *et al.* (2011), also reported that the activity of *Cestrum nucturnum* methanolic extracts act as larvicide against *Aedes aegypti* and showed outstanding activity against larvae achieving 100 % mortality in 24 hours when tested with a high concentration of 45 µg/ml. The strong correlation between Saponin content of *Balanite aegyptiaca* extract and the mortality of *Aedes aegypti* larvae exposed at 0.0014% (v/w) was demonstrated by Wiesman and Chapagain (2006).

The larvicidal activity of the chloroform extract on larvae of mosquitoes Table 5 showed high mortality after 12 and 48 hrs of exposure on *Aedes and Culex* flower and whole plant extract with the percentage mortality of 91.67 % and 90.00 with LC_{50} and LC_{90} of 1.879, 15.360 and 0.683, 72.085 respectively. This is supported by the findings of Hadjiakhoondi *et al.* (2005), who reported chloroform and methanol extracts of *Tagates minuta* against the larvae of *Anopheles stephensi* demonstrated a pronounced larvicidal effect.

In this research, we investigate the possible involvement of the enzyme Na^+/K^+-ATPase. Ions are distributed unequally in and out of the cell and this regulates the cell activities. The cell membrane is selective in allowing influx and outflux of the cell to ensure regulated action and resting potentials. Table 6 shows the atomic absorption spectroscopic data of both the treated and control group. The concentration of ions displayed reveals an uneven distribution of implicated ions with decreased concentration of sodium and corresponding increased concentration of potassium and these result in hyper relaxation, paralysis and eventually death of the larvae. These findings indicate that Na^+/K^+-ATPase are the main site of action of *Striga hermonthica* crude extract.

Conclusion

The study on the larvicidal activity of the aqueous, methanolic and chloroform flower, leaf and whole plant of *S. hermonthica* extracts against the larvae of *Aedes, Anopheles* and *Culex* mosquito showed that flower, leaves and the whole plant has larvicidal activities which could be due to the presence of secondary metabolites that possess insecticidal and larvicidal activities against the Larvae of *Aedes, Anopheles* and *Culex*

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