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Oladele O.O., Fatukasi O.I.

Medicinal Plant Research, 2018, Vol.8, No.1

Biotoxic Efficacy of Different Fractions of *Acalypha godseffiana* Oil Extract against *Callosobruchus maculatus* Infesting Stored Cowpea

Oni M.O., Ogungbite O.C., Ofuya T.I.

Medicinal Plant Research, 2018, Vol.8, No.2

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Medicinal Plant Research, 2018, Vol.8, No.3

Medicinal Plant Research 2018, Vol.8, No.3, 14-20
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Abstract The study aimed at determining the larvicidal and Insect-Growth-Regulating (IGR) potentials of n-hexane and methanolic extracts of Leaf of *Ficus vallis-choudae* against *Culex quinquefasciatus* mosquitoes in a bid to develop a cost effective and eco-friendly control lead. Collected leaves of the plants were air-dried, pulverized and sohxlet-extracted. Phytochemical screening of the crude extracts was done following standard protocols. Graded concentrations of 0.00625 to 0.05 mg/L (n-hexane extract) and 0.2 to 0.5 mg/L (methanolic extract) of the crude extract were assayed against fourth larval instar of the mosquito species. The mosquitoes were monitored from zero to 48 hours post-exposure to determine mortality. Sub lethal concentrations of 0.00625 (n-hexane) and 0.2 mg/L (methanol) were assayed for IGR activities. The phytochemical screening of crude extracts of the leaf revealed presence of seven secondary metabolites, whose presence was dependent on extraction solvent. They include flavonoids, tannins, saponins, cardiac glycosides, steroids, anthraquinones and terpenoids. After 48 hours post-exposure, analyses revealed a time and dose-dependent toxicity of the leaf extracts. The LC_{50} and LC_{90}, respectively, of the n-hexane and methanolic extracts were, 0.022 and 0.042 mg/L, and 0.355 and 0.463 mg/L, respectively. IGR studies revealed significant increase in duration of development by the sub lethal concentrations. This study revealed larvicidal and IGR potential of n-hexane and methanolic extracts of the leaf of *Ficus vallis-choudae*, and therefore, a promising lead agent for development of larvicides for mosquito vector control.
Keywords Phytochemicals; Bioassay; Toxicity; Lethal concentrations

Background

Vector-borne disease still remains and represents a great threat to public health. Among these, are those transmitted by mosquitoes, precisely, the female mosquitoes. These diseases transmitted by mosquitoes cause millions of death every year (Kamaraj et al., 2011). Several mosquitos' species belonging to the genera *Anopheles, Culex* and *Aedes* carry disease pathogens of malaria, filariasis, encephalitis, dengue and yellow fever (Hubalek and Halouzka, 1999). Mosquito-transmitted diseases have remained the major cause of morbidity and mortality in Sub-Sahara Africa (Taubes, 2000; Jang et al., 2002). For instance, there are up to 500 million clinical cases and about one million death due to malaria globally, and sub-Sahara, respectively; with Africa recording over 90% of these cases (Snow et al., 2005).

Culex quinquefasciatus is a peridomestic mosquito rarely found far from human residences and/or his activities, and feed on birds, mammals and human hosts. Having complete metamorphosis comprising of egg, larva, pupa and adult, the mosquito species has four larval instars, namely, Larval stages 1-4 (i.e., L1-L4) (Ukubuiwe at al., 2012). The mosquito species are the primary vector of lymphatic filariasis, which have remained one of the most prevalent disease widely distributed in the tropics, affecting about 120 million people worldwide, with 44 million people manifesting chronic symptoms (Bernhard et al., 2003). In recent times, increased insecticide resistance by the vector and destruction of non-target organisms in the environment have been a major drawback in the eradication of these diseases (Zaim and Guillet, 2002; Omena et al., 2007).

Plants are very rich source of alternate reservoir of bioactive chemicals that are capable of controlling disease vectors, especially, mosquitoes, hence curbing the spread of vectored diseases (Olayemi et al., 2017). Plant

pesticides have be found to be less toxic, possessing growth-delaying capabilities, easily biodegradable (Ignacimuthu, 2000) and target-specific (Sukumar et al., 1991). The phytochemicals derived from plant parts can act as larvicides and repellents against various target organisms (Pedro et al., 2014). A great number of plants in Africa, including Nigeria, have been, traditionally, observed (or noted) for their medical and pesticide properties (Okwute, 1992).

Ficus vallis-choudae, a plant widely spread in the tropical Africa including Nigeria, is one of such plants. It belongs to the Moraceae family, and has immense ethno-medicine value. The plant possess antifungal activities and extensively used in the treatment of jaundice, gastrointestinal problem and epilepsy (Adekunle, 2006). Despite these acclaimed properties of the plant, its larvicidal and growth-regulating potentials have not been investigated, this study, thus aims at elucidating these potentials systematically and updating current literature on the plant.

1 Materials and Methods

1.1 Collection and maintenance of larvae

Mosquito egg rafts were collected from stagnant water bodies in Minna, Niger State. The egg rafts were brought to the Laboratory of the Department of Biological Sciences, Federal University of Technology (FUT) Minna, Niger State. Hatched-out mosquito larvae were fed fish feed (Cuppens®) until they developed into the fourth instar (Ukubuiwe et al., 2016).

1.2 Collection, authentication and processing of leaves of *Ficus vallis-choudae*

The leaves were collected from various locations in Minna, Niger state, Nigeria (longitude $6^0$33'E and latitude $9^0$27'N). The leaves were authenticated by a Botanist in the Department of Biological Sciences, FUT, Minna, Niger State, and shade-dried in the laboratory at room temperature of 28°C for a period of three weeks.

1.3 Preparation of crude extracts of *F. vallis-choudae*

The dried leaves were weighed (50 g) and placed in the extracting flask of the Sohxlet apparatus. Two extracting solvents, n-hexane and methanol, were put in turn in the conical flask of the apparatus, for the production of n-hexane and methanol extracts of the plant, respectively. The liquid extracted was transferred into an evaporating dish, where water bath was used to dry-up the solvents. The extract was scooped and preserved in the refrigerator at 4°C until needed for bioassay (WHO, 2005; Olayemi et al., 2017).

1.4 Phytochemical screening of *F. vallis-choudae*

Qualitative phytochemical screenings of the crude extracts were carried out using standard procedures described by Trease and Evans (1989), Evans (1996) and Harborne (1998).

1.5 Preparation of stock solution and working concentrations

Working solutions for the bioassay were done according to the methods of Olayemi et al. (2017). Briefly, stock solutions of both extracts were prepared by dissolving the crude extracts in their respective solvent of extraction in the ratio 1:10 (for example, one gram of n-hexane crude extract was dissolved in 10 ml of n-hexane). This resulted in working solutions of 0.2, 0.3 and 0.4 mg/L for the methanol extract, and 0.00125, 0.0025, and 0.005 mg/L for the n-hexane extract.

1.6 Data analysis

The results obtained were expressed as mean ± Standard error of mean. Differences between mean were analyzed using analysis of variance (ANOVA), while significant differences between treatments were separated using Duncan multiple range test (DMRT), using Statistical Packages for Social Sciences (SPSS) Version 23. All decisions were taken at $p = 0.05$ using. The larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90}.

2 Results

2.1 Phytochemical screening of crude extracts of leaf of *F. vallis-choudae*

Seven (7) bioactive secondary metabolites were encountered in the Crude extracts of the leaf of the plant. They

include flavonoids, tannins, cardiac glycosides, steroids, saponins, terpenoids and anthraquinones. Five (5) of these were encountered in the n-hexane extract, while six (6) in the methanolic extract. All metabolite components were present in the former extract, except tannins and cardiac glycoside, while only anthraquinones was absent in the methanolic extract (Table 1).

Table 1 Phytochemicals components of Leaf of *Ficus vallis-choudae*

Photochemical Components	n-Hexane	Methanolic
Flavonoids	+	+
Tannins	-	+
Saponins	+	+
Steroids	+	+
Cardiac glycosides	-	+
Anthraquinones	+	-
Terpenoids	+	+
TOTAL	5	6

Note: + = represents present; - = represents absent

2.2 Toxicity of crude extracts of leaf of *F. vallis-choudae* against *Culex quinquefasciatus*

Both extracts types were found to be toxic against the fourth larval instar of the mosquito species. The toxicity of both extracts was concentration and time-dependent, i.e., greater mortality was achieved at higher concentrations and with increase in time. For the n-hexane extract, 0.025 mg/L elicited 100% mortality (30.00 ± 0.00) at 48 hours, while similar feat was obtained by 0.05 mg/L within 12 hours (Table 2).

Table 2 Mortality of *Culex quinquefasciatus* larvae exposed to n-hexane extracts of leaf of *Ficus vallis-choudae*

Time (Hours)	Extract Concentration (mg/ L)					
	Negative Control	Positive Control	0.00625	0.0125	0.025	0.05
12	$0.00\pm0.00^{a*}{}_{a**}$	$0.00\pm0.04^{a}{}_{a}$	$1.21\pm0.74^{b}{}_{a}$	$5.50\pm2.28^{c}{}_{a}$	$10.75\pm0.62^{d}{}_{a}$	30.00 ± 0.00^{e}***
24	$0.00\pm0.00^{a}{}_{a}$	$0.01\pm0.00^{a}{}_{a}$	$2.50\pm0.64^{b}{}_{b}$	$8.75\pm0.62^{c}{}_{b}$	$21.25\pm1.43^{d}{}_{b}$	-
48	$0.00\pm0.00^{a}{}_{a}$	$0.02\pm0.08^{a}{}_{a}$	$3.01\pm0.11^{b}{}_{c}$	$10.25\pm0.62^{c}{}_{c}$	$30.00\pm0.00^{d}{}_{c}$	-

Note: N = 30 larvae Positive Control = 0.00625 mg/ L n-hexane, Negative Control = Distilled water

Values are presented in mean ± standard error of mean 4 of replicates

*Values followed by the same alphabets in a column are not significantly different at $p < 0.05$ level of significance; **Values followed by the same alphabets in a row are not significantly different at $p < 0.05$ level of significance; *** - = 100% mortality attained before 24-hour exposure

Similarly, in the methanolic extract, 0.4 mg/L concentration cause greatest mortality at the 48th hour post-exposure, while similar toxicity was achieved by 0.5 mg/L before 24 hours of exposure (Table 3). Lethal concentrations of the extracts of the n-hexane and methanol leaf extracts were, respectively, 0.022 and 0.042 mg/L and 0.355 and 0.463 mg/L for the LC_{50} and LC_{90} (Table 4).

Table 3 Mortality of *Culex quinquefasciatus* larvae exposed to methanolic extracts of leaf of *Ficus vallis-choudae*

Time (hours)	Extract Concentration (mg/ L)					
	Negative Control	Positive Control	0.2	0.3	0.4	0.5
12	$0.00\pm0.00^{a*}{}_{a**}$	$0.00\pm0.10^{a}{}_{a}$	$0.50\pm0.29^{b}{}_{a}$	$2.15\pm0.31^{c}{}_{a}$	$13.75\pm0.47^{d}{}_{a}$	-***
24	$0.00\pm0.00^{a}{}_{a}$	$0.01\pm0.02^{a}{}_{a}$	$0.75\pm0.48^{b}{}_{a}$	$2.25\pm0.48^{c}{}_{a}$	$25.00\pm0.07^{d}{}_{b}$	-
48	$0.00\pm0.00^{a}{}_{a}$	$0.01\pm0.12^{a}{}_{a}$	$3.75\pm1.11^{b}{}_{b}$	$5.00\pm1.08^{c}{}_{b}$	$30.00\pm0.00^{d}{}_{c}$	-

Note: N = 30 larvae; Positive Control = 0.2 mg/ L methanol, Negative Control = Distilled water

Values are presented in mean ± standard error of mean of four replicates

*Values followed by the same alphabets in a column are not significantly different at $p<0.05$ level of significance; **Values followed by the same alphabets in a row are not significantly different at $p < 0.05$ level of significance; *** - = 100% mortality attained before 24 hour exposure.

Table 4 Lethal concentrations (mg/ L) of Leaf Extracts *Ficus vallis-choudae* against 4th instar larvae of *Culex quinquefasciatus*

Solvent	LC_{50}	LC_{90}	R^2	Regression
n-hexane	0.022	0.042	0.929	Y=2052x+3.959
Methanol	0.355	0.463	0.885	Y=368.3x-8058

Note: R^2 = coefficient of determination that shows the relationship between the extract concentration and mortality.

2.3 Growth regulatory activities of crude extracts of Leaf of *F. vallis-choudae* against *Cx. quinquefasciatus*

There was no significant ($p > 0.05$) effect of both crude extract type on survivorship of the mosquito species. Average Larval and Survivorship were, respectively, 94.59 ± 2.18 and 99.17 ± 0.54 % and 96.03 ± 1.12 and 99.67 ± 0.18 % for n-hexane and methanol extract, respectively (Figure 1).

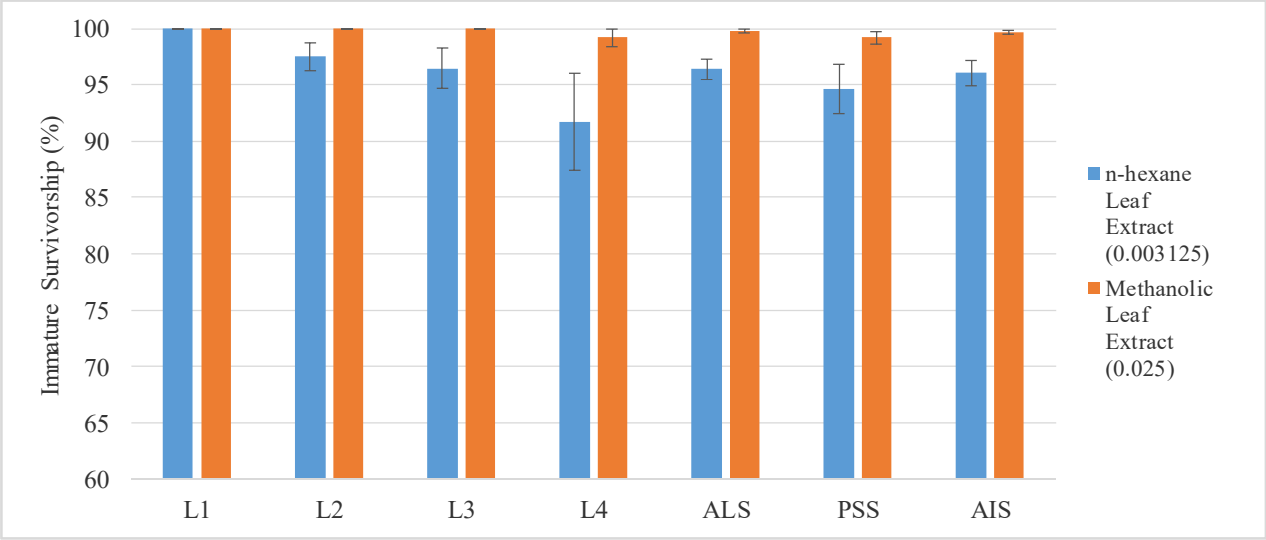

Figure 1 Effects of Sub-lethal concentrations of methanolic and n-hexane leaf extracts of Ficus vallis-choudae on Survival rate (%) of Immature Life Stages of Culex quinquefasciatus mosquitoes L1-4 = Larval Stages, ALS= Average Larval Survivorship, PSS= Pupal Stage Survivorship, AIS = Average Immature Survivorship

Duration of development, on the other hand, was significantly ($p < 0.05$) affected by the plant extracts. Total larval duration (TLD) was delayed from 7.24 ± 0.21 days in the negative control to 10.63 ± 0.16 and 10.63 ± 0.16 days, respectively, in n-hexane and methanol leaf extracts. Similarly, pupal stage duration (PSD) of development was slowed increased from 1.24 ± 0.08 days in the negative control to 2.65 ± 0.04 and 2.79 ± 0.09 days, respectively, in n-hexane and methanol leaf extracts (Table 5).

Table 5 Effects of Sub-lethal concentrations of methanolic and n-hexane leaf extracts of *Ficus vallis-choudae* on duration of development (Days) of *Culex quinquefasciatus* mosquitoes

Solvent (mg/ L)	**Larval Instars**				**TLD**	**PSD**	**TID**
	L1	**L2**	**L3**	**L4**			
Negative Control (0.00)	1.41±0.03^{a*}	1.58±0.02^{a}	1.88±0.05^{a}	2.37±0.11^{a}	7.24±0.21^{a}	1.24±0.08^{a}	8.48±0.29^{a}
n-hexane Leaf Extract (0.003125)	2.28±0.02^{b}	2.72±0.03^{b}	2.37±0.07^{b}	3.26±0.04^{b}	10.63±0.16^{b}	2.65±0.04^{b}	13.28±0.20^{b}
Methanolic Leaf Extract (0.025)	2.60±0.08^{b}	2.14±0.13^{b}	2.56±0.11^{b}	3.00±0.06^{b}	10.30±0.38^{b}	2.79±0.09^{b}	13.09±0.47^{b}

Note: Values are presented in mean ± standard error of mean of four replicates. TLD =Total Larval Duration, PSD = Pupal Stage Duration, TID = Total Immature Duration

* Values followed by the same alphabets in the similar column are not significantly different at $p < 0.05$ level of significance

These led to a significant increase in Total Immature Duration (TID) of development from 8.48 ± 0.29 days to 13.28 ± 0.20 and 13.09 ± 0.47 days, respectively, and in n-hexane and methanol leaf extracts (Table 5).

3 Discussions

This study revealed the presence of seven secondary metabolites; these were flavonoids, tannins, cardiac glycosides, steroids, saponins, terpenoids and anthraquinones. These bioactive compounds have been reported to possess therapeutic (Lawan et al., 2008), toxicity (Shaalan et al., 2005) and larvicidal effects (Pedro et al., 2014) on mosquitoes. There were disparity in the numbers of these phytochemical constituents encountered in the two extract types (five in n-hexane extract and six in methanolic extract). These differences could be due to solvent of extraction (Raghavendra et al., 2011). El Tayeb et al. (2009) had observed variations in the phytochemical constituents of the plant part. Earlier, Olayemi et al. (2017) had reported similar observation in the numbers of phytochemical constituents in *F. sur* (a close relative) using similar solvents of extraction, although, variation in phytochemical constituents of the extract types were encountered. This difference could have been due to the plants species (Kamaraj et al., 2011).

In the present study, the two extract types of leaf of *Ficus vallis-choudae* (n-hexane and methanolic) were found to be toxic to fourth larval instar of the mosquito species; this effect was, largely, concentration- and time-dependent. As concentration of extracts and duration of exposure of the mosquito to the extracts increase, mortality also increases. Imam and Tajuddeen (2013) and Olayemi et al. (2013; 2017) observed similar trends. This could be due to increased toxicity associated with increase in concentration of the extract (Shaalan et al., 2005; Kishore et al., 2011).

Further, the n-hexane extract of the leaf of *Ficus vallis-choudae* was more toxic than the methanolic extract: as the highest value tested for methanol was 10 times the highest for n-hexane. This could have been due to polarity of the solvents used (Kishore, 2011). Lethal concentrations of the extracts of the n-hexane and methanol leaf extracts were, respectively, 0.022 and 0.042 mg/L and 0.355 and 0.463 mg/L, respectively, for the LC_{50} and LC_{90}. These values were lower than those reported for *Carica papaya* (Olayemi et al., 2013), *Jatropha curcas* (Olayemi et al., 2014) and propolis (Adeniyi et al., 2017). Although, with higher LC_{50} values, the extracts of this plant (*Ficus vallis-choudae*) had lower LC_{90}s than *F. sur* and *Adansonia digitata* (Olayemi et al., 2017).

In the present study, though, survivorship of immature life stages of *Cx. quinquefasciatus* was not affected by sub-lethal concentrations of the plant extracts, duration of development was significantly increased. This finding is epidemiologically important as delay or elongation of duration of development would ensure suppressed metamorphosis, with its attendant reduction in population below threshold required to sustain disease transmission (Olayemi et al., 2013). The plant extracts could have delayed development by acting as a growth hormone suppressant of (MacRae, 2010), could have disrupted feeding activities by mosquito species. The latter have resultant effects on the accumulation of threshold-biomass required for metamorphosis of life stages (Timmerman and Briegel, 1993).

4 Conclusions

This study has revealed that crude extracts of the leaf of *F. vallis-chaude* contains secondary metabolites with biomedical importance. The efficacies of these extracts are dependent on solvent of extraction, duration of exposure to mosquitoes and test concentration of the extracts. More so, the extracts were toxic to *Cx. quinquefasciatus* mosquitoes, acting as a larvicide and a growth regulator. Therefore, this plant could be a viable lead agent in the development of natural bio-pesticide for the control of mosquito disease vector.

Further studies, however, are advocated to evaluate the efficacies of fractionated portions of these extracts and other extract types of the leaf against the mosquito species. Further, mosquitocidal potency of other parts of the plant should be investigated and toxicity of these fractions against non-target aquatic and terrestrial organisms appraised.

Author's contributions

OJL, OIK and UAC conceived and designed the experiment. OJL, AA, OJO and SOM performed the experiments. OIK, UAC and AKA analysed the data. OJL and UAC wrote the first draft of the manuscript. OIK and AKA contributed to writing of the manuscript. All Authors agreed with manuscript results and conclusion. All authors read and approved the final manuscript.

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