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Original Article

Momordica charantia L. extracts against *Aedes aegypti* larvae

Extratos de *Momordica charantia* L. sobre larvas de *Aedes aegypti*

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Abstract

Mormodica charantia (Curcubitaceae) is a plant with great medicinal potential, also used as an alternative of mosquitoes control as demonstrated by previous studies. We evaluated the larvicidal activity of crude extracts of ethyl acetate, methanol and hexane from flowers and fruits of *M. charantia* against *Aedes aegypti* (Culicidae). Flowers and fruits were macerated in methanol, ethyl acetate and hexane. Bioassays were performed with application of the extracts at final concentrations of 1 - 200 µg/mL in the middle of the third instar larvae of *A. aegypti* (L3). The results showed high toxicity to ethyl acetate extracts from flowers and fruits at concentrations of 200 µg/mL and 100 µg/mL, with 97% and 87% of larvae mortality (L3), respectively. Hexane extract demonstrated low toxicity, while methanol extract exhibited 78% larval mortality. The data suggested that the ethyl acetate extracts of flowers and fruits of *M. charantia* can effectively contribute to larvicidal activity. In addition, purification of *M. charantia* extracts may lead to a promising larvicidal activity to control the *A. aegypti* population.

Keywords: larval mortality, control population, larvicidal activity, Curcubitaceae, Culicidae.

Resumo

Mormodica charantia (Curcubitaceae) é uma planta com grande potencial medicinal, sendo também uma alternativa no controle de mosquitos conforme demonstrado por estudos prévios. Avaliou-se a atividade larvicida dos extratos brutos de acetato de etila, metanólico e hexânico das folhas, flores e frutos de *M. charantia* no *Aedes aegypti* (Culicidae). Folhas, flores e frutos foram macerados em metanol, acetato de etila e hexano. Os bioensaios foram realizados com aplicação dos extratos nas concentrações finais de 1-200 µg/mL no meio de criação das larvas de terceiro estádio de *A. aegypti* (L3). Os resultados obtidos apontaram alta toxicidade para os extratos de acetato de etila das flores e frutos nas concentrações de 200 µg/mL e 100 µg/mL com mortalidade em L3 de 96,7% e 87%, respectivamente. Baixa toxicidade para o extrato hexânico e o extrato metanólico apresentou mortalidade de 78% larval. Os dados sugerem que os extratos de acetato de etila das flores e frutos de *M. charantia* podem contribuir efetivamente para atividade larvicida no controle da população de *A. aegypti*.

Palavras-chave: mortalidade larval, controle da população, atividade larvicida, Curcubitaceae, Culicidae.

1. Introduction

Plants are a rich source of chemical bioactive compounds that can act towards insect control. The diversity of Brazilian flora presents immense potential for compounds that can act as insecticides (Bezerra et al., 2014). Traditional knowledge of the use and efficacy of plants has contributed significantly to the learning of their properties, which has attracted interest among researchers in several fields of study (Dutra et al., 2016). The plant species *Momordica charantia* L., of the family Cucurbitaceae, is widely farmed in tropical and subtropical countries. *M. charantia* is of African origin and can be found in several countries, including Brazil, where it has become popular under the names *Melão-de-São-Caetano*, *Erva de lavadeira*, *Fruto de cobra* and *Erva de São Vicente*, among others (Zocoler et al., 2006).

It is known as a traditional medicinal fruit and represents an alternative for studies on controlling disease-causing vectors, since this plant presents a range of therapeutic properties, including anticancer, antiviral, anti-inflammatory, hypoglycemic, antimalarial and other activities (Jia et al., 2017). Moreover, this species has shown larvicide activity against three species of mosquitos: *Anopheles stephensi* (Liston, 1901),

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Culex quinquefasciatus (Say, 1823) and *Aedes aegypti* (Linnaeus, 1762) (Singh et al., 2006; Pari et al., 2020).

With the increasing epidemic cases of dengue fever, chikungunya and zika, the *A. aegypti* mosquito has become an important public health issue, since it presents the greatest dispersion in urban areas of the world (Gould et al., 2017). Moreover, this species is the main vector of the viruses which cause these diseases (Gould et al., 2017). Several factors have promoted recurrence of dengue epidemics and other diseases transmitted by *A. aegypti*, such as the proliferation of mosquitoes, rapid demographic growth associated with intensive and disorganized urbanization and inadequate infrastructure, among other factors (Mendonça et al., 2009).

Efforts toward the prevention of arboviral diseases transmitted by the *A. aegypti* mosquito are aimed at the reduction of vector density through epidemiological surveillance and vector-control strategies (Brasil, 2009). Although it is recognize the importance of social participation and community involvement to avoid high levels of insect infestation, the chemical control is an important strategy in endemic areas that can be accomplished through the application of chemical pesticides (larvicides and insecticides) (Brasil, 2009). However, the indiscriminate overuse of those pesticides gradually increases the ability of the *A. aegypti* mosquito to tolerate synthetic chemical insecticides, impairing their effectiveness (Naqqash et al., 2016).This is a factor that associated with the need for less toxic products for both human health and the environment, increases the interest for exploring new approaches to higher effectiveness in the control of *A. aegypti* (Pavela, 2016; Benelli, 2018; Muangmoon et al., 2018).

Rahuman and Venkatesan (2008) highlight that plant extracts and essential oils can indeed be alternative sources for controlling mosquito larvae, as well as constituting a rich source of bioactive and biodegradable compounds into a nontoxic product potentially suitable for use in mosquito larvae control.

Plants offer an extraordinary diversity of metabolites with proven efficacy against insects of medical and veterinary importance, as well as other insect species considered agricultural pests (Formentini et al., 2016; Panchal and Tiwari, 2017; Spochacz et al., 2018; Cruz et al., 2019).

Thus, insecticidal plant extracts have emerged as a subject of study and have been evaluated as an alternative for integrated management of crop pests. They may also be a good alternative for controlling vectors of diseases (Pavela, 2016).

The present study contributes to a promissing alternative for combating the causative agent of urban yellow fever, dengue fever, chikungunya and zika through the use of *M. charantia* extracts. This plant has gained great importance due to its medicinal properties, which has led to the need for further studies assessing additional benefits, such as the action of *M. charantia* leaf, fruit and flower extracts on the larvae of *A. aegypti.*

2. Material and Methods

2.1. Botanical material

Mormodica charantia was collected in the municipality of Valença, state of Rio de Janeiro, southeastern Brazil (22° 14' 44" S; 43° 42' 01"W), which is at an altitude of 560 m and covers an area of 1308.1 km^2. The botanical voucher was collected during the mornings of the months of October and December 2015.

The botanical voucher was identified by Dr. Ana Carla Pinheiro Lima, and the sample was deposited in the herbarium of the University of Vassouras, Vassouras, Rio de Janeiro, Brazil, registered under the number HUSSCUR001.

2.2. Extraction

Flowers and fruits (EFF) of *M. charantia* were used in combination to obtain the plant extracts. The plant material was washed with distilled water and dried at room temperature. After drying, the material was fragmented and then ground to prepare the extracts. The flowers and fruits (74 g) were macerated together using the following solvents of increasing polarity: hexane (Isofar) (400mL), ethyl acetate (Quimex) (400 mL) and methanol (Vetec) (400 mL; 1:1). Thus, hexane, ethyl acetate and methanol extracts were obtained, respectively. All the materials were prepared in dark-glass flasks, with occasional agitation twice a week for a period of 20 days. After maceration, the extracts were filtered and concentrated in a rotary evaporator, under reduced pressure at 40 °C. The product was then stored in a dark glass flask and placed in a fridge at 12°C and 25% relative humidity (RH).

2.3. Bioassays

A. aegypti eggs were obtained from the Sentinel Operational Unit of Mosquito Vectors (Nosmove), Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. Bioassays were conducted at the Vector Insect Laboratory, University of Vassouras, Vassouras, Rio de Janeiro, Brazil. For these tests, the eggs were analyzed regarding their viability and placed for hatching in containers filled with mineral water previously heated to 28 °C, and then fish feed was added (Alcon Guppy®). The containers with the eggs were kept in a biochemical oxygen demand (BOD) climatized chamber at 27 ± 1 °C and 70 ± 10% RH. After hatching, the third-stage larvae (L3) were separated to perform the bioassays.

The hexane (HEX), ethyl acetate (AcOEt) and methanol (MeOH) crude extracts (EFF) were diluted with dimethyl sulfoxide (DMSO) in the following proportions: HEX: DMSO (1:3), AcOEt: DMSO (1:3) and MeOH: DMSO (1:3), respectively, obtaining final concentrations of 1, 10, 50, 100 and 200 µg/mL. Subsequently, these solutions were applied (µL: mL) to the larval rearing environment in glass containers containing mineral water (20 mL) in a ratio of 1:1.

Bioassays were conducted on 20 third-stage larvae (L3) per testing group and on a control group (without substance and dilution solvent), including also a witness control group (without substance and with dilution solvent). The experiments were performed in triplicate

(R1, R2 and R3), thereby totaling 60 larvae per group, with three repetitions. After this procedure, the insects received a normal diet (0.3 mg fish feed for each larva) and were kept in a BOD climatized chamber at 27 ± 1 °C and 70 ± 10% RH. They were observed for 25 days with respect to development and mortality. Bioassays were conducted according to the methodology of Maleck et al. (2013), adapted from WHO (2005).

2.4. Statistical analysis

The biological bioassay results were subjected to the Tukey test using GraphPad Prism version 6.0. One-criterion analysis of variance (ANOVA) was also applied, and this was considered significant for P<0.01 (Sokal and Rohlf, 1979). Standard deviation was calculated based on the mean values of the experiments. The statistical analysis to calculate LC_{50} followed the trimmed Spearman-Karber methodology (Hamilton et al., 1977).

3. Results

Extraction from the flowers and fruits (EFF) of *M. charantia* yielded 330 mg of AcOEt extract, 290 mg of MeOH extract and 42 mg of HEX extract.

The AcOEt extract used at a concentration of 100 µg/mL demonstrated behavioral alterations among larvae, with low mobility and lethargy within 24 hours of larval treatment (L3). Concerning the mosquito development cycle, the larval period was reduced by four days (7±1.2 days; P<0.0001) at a concentration of 100 µg/mL when compared with the testimony control group (11±2 days). In turn, the L3-adult period was reduced by three days (10±1.8 days; P<0.001) (as shown in Table 1 (1A)) compared with the testimony control group (13±1.9 days). The same extract presented 96.7% (P<0.001) and 86.7% (P< 0.001) larval mortality (L3) at concentrations of 200 µg/mL and 100 µg/mL, respectively, for up to 48 hours of treatment (as shown in Table 1 (1B)).

This extract revealed an LC_{50} value of 37.2 µg/mL. In addition, bioassays with the AcOEt extract demonstrated larval viability (L3-L4) of only 3.3% (200 µg/mL) and 13.3% (100 µg/mL).

At lower concentrations, the AcOEt extract at 50 µg/mL reduced the larval period by four days (8.1±1.7 days; P<0.0001) as compared with the control testimony (12.5±3.1 days) (as shown in Table 2 (2A)). The time required for L3-to-adult development showed a reduction at concentrations of 10µg/mL (12.9±2.8 days; P<0.1) and 50 µg/mL (10.8±1.9 days; P< 0.0001), in relation to the control testimony (15.3±3.3 days) (as shown in Table 2 (2A)). The same extract showed low mortality among L3 larvae at concentrations of 1 µg/mL and 10 µg/mL (6.7% and 10%), respectively (as shown in Table 2 (2B)). Similarly, mortality was low in the case of L4 larvae (10 and 5%) (as shown in Table 2 (2B)). At the concentration of 50 µg/mL, the extract presented moderate mortality for L3 (40%) (P<0.01), but low mortality for L4 and pupae (13.3% and 3.5%), respectively (as shown in Table 2 (2B)).

Regarding the larval development period, the MeOH extract at concentration of 200 µg/mL (8.5 ± 0.9 days; P<0.0001) reduced the larval period, in comparison with the testimony control (11.8 ± 1.9 days). Besides, the pupal period at concentrations of 100 µg/mL (2.1 ± 0.4 days; P<0.01) and 200 µg/mL (4.5 ± 2.2 days; P<0.0001) were extended when compared with the testimony control group (1.7 ± 0.6 days) (as shown in Table 3 (3A)).

Twenty-four hours after treatment, the larvicidal activity of the MeOH extract of *M. charantia* resulted in low mobility and lethargy among the larvae. Mortality of L3 larvae reached 70% (P<0.001) at the concentration of 200 µg/mL (as shown in Table 3 (3B)), with 20% viability (P<0.01) for L3-adult development, resulting from larval disintegration and thus reducing the chances of larval emergence. This extract exhibited low toxicity at the concentration of 100µg/mL, with only 1.7% L3 larval mortality, 10% L4 larval mortality, and 3.3% pupal mortality

Table 1. Duration of development (A) and mortality (B) of *Aedes aegypti* in L3 larvae treated in a rearing environment with crude ethyl acetate extract (AcOEt) from the flowers and fruits of *Momordica charantia.*

Application	Larval (days)			Pupal (days)			L3-Adult (days)		
1A	**X ± SD**	**R**		**X ± SD**	**R**		**X ± SD**	**R**	
Control	12.2±2^{a}	4-15		1.7±0.5^{a}	1-3		13.5±1.8^{a}	5-16	
Testimony	11±2^{b}	6-14		2.3±0.7^{b}	1-4		13±1.9^{ab}	9-15	
100 µg/mL	7±1.2^{c****}	5-8		2.8±0.9^{b}	2-5		11±1.8^{c***}	8-13	
200 µg/mL	10±1.4^{bc}	9-11		3±1.4^{b}	2-4		13±2.8^{ab}	11-15	
1B	**L3**			**L4**			**Pupa**		
	X ± SD	**R**	**%**	**X ± SD**	**R**	**%**	**X ± SD**	**R**	**%**
Control	0 ± 0^{a}	0	0	0	0	0	0.3 ± 0.5	13-13	1.7
Testimony	0 ± 0^{ab}	0	0	0	0	0	0	0	0
100 µg/mL	17 ± 1.0^{c***}	2-7	86.7	0	0	0	0	0	0
200 µg/mL	19 ± 1.0^{d***}	2-5	96.7	0	0	0	0	0	0

Experiments with 20 *A. aegypti* larvae (L3) for each test and control group were performed in triplicate and with three repetitions. Mean and standard deviation (X ±SD). Range (R). Values followed by the same letter do not present significant differences. Significance levels through the Tukey test, represented as ****P<0.0001; ***P<0.0001.

Table 2. Duration of development (A) and mortality (B) of *Aedes aegypti* in L3 larvae treated in a rearing environment with crude ethyl acetate extract (AcOEt) from the flowers and fruits of *Momordica charantia.*

Application	Larval (days)		Pupal (days)		L3-Adult (days)	
2A	X ± SD	R	X ± SD	R	X ± SD	R
Control	11.7±4.8^{a}	2-23	2.8±0.7^{a}	1-5	14.4±4.9^{a}	5-25
Testimony	12.5±3.1^{ab}	7-19	2.9±0.6^{ab}	2-4	15.3±3.3^{ab}	9-22
1 µg/mL	13.1±4.4^{ab}	5-22	2.4±0.8^{ac*}	1-4	15.7±4.5^{ab}	8-24
10 µg/mL	10±1.9^{ac**}	7-15	3±1.5^{ab}	1-9	12.9±2.8^{ac*}	8-21
50 µg/mL	8.1±1.7^{c****}	5-11	2.6±0.8^{ab}	1-4	10.8±1.9^{d****}	8-14

2B	L3			L4			Pupa		
	X ± SD	R	%	X ± SD	R	%	X ± SD	R	%
Control	0.3 ± 0.5^{a}	1-1	3.3	0.3 ± 0.5^{a}	1-1	1.7	0.3 ± 0.5^{a}	1-1	1.7
Testimony	0.3 ± 0.5^{ab}	1-1	1.7	0^{a}	0-0	0	1 ± 0^{a}	1-1	5
1 µg/mL	1 ± 1^{ab}	1-3	6.7	2 ± 2^{a}	1-4	10	0^{a}	0	0
10 µg/mL	2 ± 3^{ab}	1-5	10	1.2 ± 1^{a}	1-2	5	0.3 ± 0.5^{a}	1-1	2
50 µg/mL	8 ± 4^{c**}	3-12	40	3 ± 0.5^{a}	2-3	13.3	0.3 ± 0.5^{a}	1-1	3.5

Experiments with 20 *A. aegypti* larvae (L3) for each test and control group were performed in triplicate and with three repetitions. Mean and standard deviation (X ±SD). Range (R). Values followed by the same letter do not present significant differences. Significance levels through the Tukey test, represented as ****P<0.0001; **P<0.01; *P<0.1 vs testimony control AcOEt:DMSO (1:3).

Table 3. Duration of the development (A) and mortality (B) of *Aedes aegypti* in L3 larvae treated in a rearing environment with crude methanol (MeOH) extract from the flowers and fruits of *Momordica charantia.*

Application	Larval (days)		Pupal (days)		L3-Adult (days)	
3A	X ± SD	R	X ± SD	R	X ± SD	R
Control	13.2±1.9^{a}	5-16	1.7±0.6^{a}	1-3	14.5±1.8^{a}	6-17
Testimony	11.8±1.9^{b}	6-14	1.6±0.6^{b}	1-4	13.5±1.8^{b}	7-16
100 µg/mL	11.2±1.9^{b}	7-13	2.1±0.4^{c**}	2-4	13.4±1.7^{b}	9-15
200 µg/mL	8.5±0.9^{c****}	7-11	4.5±2.2^{d****}	1-7	13±2.5^{b}	9-15

3B	L3			L4			Pupa		
	X ± SD	R	%	X ± SD	R	%	X ± SD	R	%
Control	0^{a}	0	0	0^{a}	0	0	0.3 ± 0.5^{a}	13-13	1.7
Testimony	0^{ab}	0	0	0^{ab}	0	0	0^{ab}	0	0
100µg/mL	0.3 ± 0.5^{ab}	1-1	1.7	2 ± 3^{ab}	6-6	10	0.3 ± 0.5^{ab}	1-1	3.3
200µg/mL	13 ± 4^{c***}	1-6	70	1 ± 0^{ab}	1-2	8.3	1 ± 1^{ab}	2-2	1.7

Experiments with 20 *Ae. aegypti* larvae (L3) for each test and control group were performed in triplicate and with three repetitions. Mean and standard deviation (X ± SD). Range (R). Values followed by the same letter do not present significant differences. Significance levels through the Tukey test, represented as **** P<0.0001; ***P<0.001; **P <0.01 vs testimony control MeOH:DMSO (1:3).

(as shown in Table 3 (3B)). Furthermore, the MeOH extract presented LC_{50}= 129.6 µg/mL.

The results indicated that the HEX crude extract showed low larval toxicity at a concentration of 100 µg/mL and resulted in 78-90% larval emergence. Considering the development period, these results were statistically similar to those obtained from the testimony control group.

4. Discussion

Diseases transmitted by mosquitoes are a threat to human health. There are many strategies to control mosquitoes like *A. aegypti* and its immatures forms (Brasil, 2009). However, the synthetic chemical insecticides currently in use have some disadvantages. Some factors such as vector resistance, toxicity to humans and non-target organisms drive the interest in exploring new control alternatives (Pavela, 2016; Benelli, 2018).

Plants are rich sources of resource for biologically active substances that show a potential to control *A. aegypti*, being considered attractive alternatives to the conventional chemical insecticides (Muangmoon et al., 2018).

In some regions of Africa, plant-based methods such as burning raw materials, crude extracts, and oil preparations have demonstrated repellency against mosquitoes and

provided protection for humans. In rural communities, these traditional methods are accessible and easily available (Pavela and Benelli, 2016). Many studies have documented the effectiveness of plant extracts and their isolated substances in controlling *A. aegypti*. In this context, Azevedo et al. (2019) evaluated the larvicidal activity of extracts from 16 native plants from the Araripe National Forest, Ceará, Brazil. Among the plants that were evaluated, the ethanolic extract of *Ocotea sp*. was the most efficient against *A. aegypti*, presenting 100% larval mortality in all tested concentrations. In a study conducted by Cruz et al. (2019), the steroidal alkaloid solasodine, isolated from the fruit of *Solanum paludosum*, caused 63% mortality of the 4th instar larvae at a concentration of 150 µg/mL.

Regarding the insecticidal activity of *M. charantia,* Pari et al. (2020) studied the activity of the ethanolic extract of *M. charantia* seed against the immature forms of *An. stephensi*, *C. quinquefasciatus* and *A. aegypti* and the results in the third instar showed LD_{50} = 246.757 ppm, LD_{50} = 239.018 ppm and LD_{50} = 228.001 ppm, respectively.

Singh et al. (2006) reported that this plant revealed larvicidal activity against three mosquito species: *An. stephensi, C. quinquefasciatus* and *A. aegypti.* Maurya et al. (2009) evaluated the larvicide activity of *M. charantia* fruit against *An. stephensi* and *C. quinquefasciatus* in petroleum ether, carbon tetrachloride and methanol extracts. The methanol extract presented activity against *An. stephensi* (LD_{50}=142.82 µg/mL, LD_{90}=524.54 µg/mL) and against *C. quinquefasciatus* (LD_{90}=579.93 µg/mL). This means that higher concentrations of the methanol extract of *M. charantia* fruit would produce a more potent larvicidal activity, as demonstrated by the study conducted by Subramaniam et al. (2012), where the larvicide activity of *M. charantia* leaves was tested against *An. stephensi*in the four larval and pupal stages. They found different activities for the methanol extract between the various stages: 1st instar, LD_{50}=93.45 µg/mL; 2nd instar, LD_{50}=123.74 µg/mL; 3rd instar, LD_{50}=167.17 µg/mL; 4th instar, LD_{50}=216.15 µg/mL; and pupae, LD_{50}=256.66 µg/mL.Methanol extract at a concentration of 100 µg/mL presented low toxicity, while at 200 µg/mL a moderate toxicity was observed. Similar data were obtained from Rahuman and Venkatesan (2008) working with the Cucurbitaceae family, who have demonstrated the existence of larvicide activity against fourth-stage *A. aegypti* larvae with a methanol extract (LD_{50}=199.14µg/ mL, LD_{90}=780.10 µg/mL), although in the current study an LD 50 was obtained at a lower concentration.

Kamaraj and Rahuman (2010) found that ethyl acetate extract from the leaves of five plant species of the family Cucurbitaceae which were tested, including *M. charantia*, produced high mortality (L4) at a concentration of 500 µg/mL against *Culex gelidus* and *C. quinquefasciatus*. This result was similar to that of the present study regarding the ethyl acetate extract from *M. charantia* flowers and fruits at concentrations of 100 µg/mL and 200 µg/mL (87% and 97%, respectively). It is important to note that the ethyl acetate extract of flowers and fruits showed the same activity at lower concentrations.

On the other hand, the hexane extract of *M. charantia* did not show toxicity for L3 larvae and only a low toxicity for L4 larvae and pupae was observed. According to the work reported by Kamaraj and Rahuman (2010), the hexane extract presented low toxicity against *C. gelidus* and *C. quinquefasciatus*. Singh et al. (2006) demonstrated that the hexane extract of *M. charantia* fruits had better larvicidal activity than the crude aqueous extract, and that *An. stephensi* larvae were more susceptible than *C. quinquefasciatus* and *A. aegypti* larvae. The difference in results can be explained based on the chemical composition of plants which may vary according to environmental factors such as soil type, humidity, solar irradiation, wind, temperature and atmospheric pollution, among others (Barreto, 2005).

The ethyl acetate extract of *M. charantia* demonstrated toxicity against *A. aegypti* larvae, thus confirming the efficacy of this species of the Cucurbitaceae family as a source of active natural plant products and its importance as a potential new larvicide for controlling the mosquito vector of dengue virus, zika, chikungunya, and urban yellow fever.

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