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Toxicity of Mexican native plant extracts against larvae of *Aedes aegypti* (Diptera: Culicidae)

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PEER REVIEW

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Comments

This is a well written paper which presents valuable information that *A. mexicana* and *P. perniciosum*, have toxic effect on *Aedes* mosquitoes larvae and they may be employed in control programs against these dengue vectors. Their toxicity against the larvae of the mosquitoes varied according to the solvent used.
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ABSTRACT

Objective: To evaluate five indigenous Mexican plants [*Hippocratea excelsa*, *Hippocratea celastroides*, *Argemone mexicana* (*A. mexicana*), *Tagetes lucida*, and *Pseudosmodingium perniciosum* (*P. perniciosum*)] toxicity against the fourth instar larvae of the dengue primary vector, *Aedes aegypti* (*A. aegypti*).

Methods: Each plant part was treated successively with hexane, ethyl acetate, acetone, and methanol to extract potential active components of the plants against the dengue vector.

Results: There was a range of toxicity at 24 or 48 h post-exposure for the different plant parts and organic solvent used (LC_{50} values ranged between 20 and 890 μg/mL). Extracts from seeds of *A. mexicana* (hexane washing with methanol and acetone) and stem-bark of *P. perniciosum* (hexane) showed highest toxicity to *Ae. aegypti* larvae at 48 h post-exposure (LC_{50} values were 80, 50, and 20 μg/mL, respectively), thus making them potential candidates as biolarvicides. Efforts are on-going to characterize the bioactive components of the extracts, through chromatography, for their use as biological tools for the control of the primary dengue vector.

Conclusions: *A. mexicana* and *P. perniciosum* are good candidates to combat the dengue vector, *Ae. aegypti*, as they were highly toxic to the larvae.

KEYWORDS
Aedes aegypti, Dengue, Larvicidal activity, Plant organic extracts

1. Introduction

Dengue is a viral disease transmitted by *Aedes* mosquitoes and constitutes a serious public health threat worldwide[1-5]. The control of the dengue primary vector, *Aedes aegypti* (*Ae. aegypti*), stems mostly on chemical insecticides. The wide spread of insecticide resistance has reduced the ability of insecticides to control mosquito vectors[2,6-8].Thus, the search for new control strategies that can tackle insecticide resistance or reduce the use of such chemicals in insect vectors are desperately needed. Plants usually produce compounds which protect them from insects, and these compounds have detrimental effect on the development of the

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insects[9,10]. These observations have therefore encouraged studies to evaluate Mexican native plants against the dengue primary vector.

One of the effective methods to control insect vectors is the prevention of mosquito breeding through the use of easily biodegradable insecticides. Much of the research against *Ae. aegypti* mosquitoes have focused on by-products of plants already utilized for economic gain or on already recognized medicinal plants[11-14]. The use of botanical insecticides has been intensified over the past decades due to their bioactive compounds, which leave no residues in the environment; hence, it's suitable for integrated vector management programs (IVMP). Traditionally, IVMP have been based on two components: chemical control (temephos as larvicide and organophosphates and pyrethroids as adulticides applied by ultra-low volume space spraying) and community contribution to remove the water in artificial containers that serve as breeding sites for the mosquitoes. However, dengue is associated to the lowest socio-economical strata of the endemic (developing) countries worldwide where the community lacks a culture of participation[15,16].

In Mexico, dengue fever has increased significantly in recent years and there are reports of resistance of *Ae. aegypti* to chemical insecticides[17-19]. Nowadays, the application of chemical insecticides is still a major tool that Mexican Health Secretariat has been using against the dengue primary vector. In Mexico, there are several reports on the use of promising biological agents to control *Ae. aegypti*[11,17]. However, most of the natural enemies of the *Aedes* mosquitoes incriminated in suburban and urban transmission areas are at experimental level. In addition, many reports have been published on the toxicity of botanical larvicides against *Ae. aegypti*[11,12,20,21].

In this study, five indigenous Mexican plants [*Hippocratea excelsa* (*H. excelsa*), *Hippocratea celastroides* (*H. celastroides*), *Argemone mexicana* (*A. mexicana*), *Tagetes lucida* (*T. lucida*), and *Pseudosmodingium perniciosum* (*P. perniciosum*)] were evaluated for its toxicity against the fourth larval instar of the dengue primary vector, *Ae. aegypti*. All these plants are easily available and have medicinal values. However, there is not known information on their larvicidal potentials against *Ae. aegypti*, except for the seed of *A. mexicana* which had been signaled as chemosterilant agent against *Ae. aegypti*[13,22]. The accessibility to indigenous plant material will enhance their use among the affected population as they have been proved to be efficient against the disease vector mosquitoes. In addition, the plants can be grown by rural or semi-urban communities which will provide sustainable and relatively cheap mosquito control.

2. Materials and methods

2.1. Plants

Selected fresh plant specimens of *H. excelsa* Kunth (Hippocrateaceae) and *H. celastroides* Kunth (Hippocrateaceae) were collected in March 2009 from the community of Iguala (18°24' N, 99°32.15' W) in the state of Guerrero. *A. mexicana* (Papaveraceae) was collected in May 2008 from the community of San Felipe Ixtacuixtla (19°19' N, 98°22' W) in the state of Tlaxcala, and *T. lucida* Cav. (Compositae) was collected between June and July 2009 from the communities of Calpulalpan (19°33' N, 98°35' W) and Nanacamilpa (19°28' N, 98°30' W) in the State of Tlaxcala. *P. perniciosum* (Anacardiaceae) was collected in September 2009 from the communities of Buenavista de Cuéllar (18°29' N, 99°27' W) in the state of Guerrero. Voucher specimens of each taxonomically identified plant were deposited at the Herbario Nacional de México in the Instituto de Biología of the Universidad Nacional Autónoma de México.

2.2. Organic extracts

Selected parts of the plants: roots, stems, leaves, flowers, seeds, and stem-bark, freshly collected were dried and each portion (⩽ 500 g) grinded through two consecutive maceration processes (each one for three days). The solvents used to extract bioactive compounds from plants were hexane, ethyl acetate, acetone, and methanol. Plants crude extracts were also concentrated and stored at -4 °C until tested. Stock solutions (500 μg/mL) of each extract containing 1% dimethylsulphoxide (Aldrich, Milwuakee, WI, USA) were prepared. Three serial dilutions 250, 125, and 50 μg/mL in distilled water were then prepared from the stock solution.

Preliminary phytochemical screening of plants with better activity was carried out to identify chemical groups of substances such as alkaloids, flavonoids, coumarins, saponins, tanins, cardiotonics glycosides, and sterols and/or terpenes[23].

2.3. Mosquitoes

Ae. aegypti eggs were collected from the state of Guerrero, Mexico by the personnel of the Mexican Health Secretariate. Briefly, pieces of filter paper with the eggs attached were submerged into 500 mL of dechlorinated water for 30 to 60 min to allow the eggs to hatch into larvae. The larvae were reared under insectary conditions at (27 ± 2) °C, 70% ± 10% relative humidity and 14 h-10 h light-dark photoperiod. They were daily fed *ad libitum* with fish food until pupation. The pupae were collected and placed inside cages, where adults were fed with a 10% sucrose-honey solution. Three day old female mosquitoes were placed in metal cages with a surgical stockinet sleeve and fed with rabbit blood for 2 h every four days as previously described[24,25]. The gravid mosquitoes laid their eggs and subsequent generations (F_1 and F_2) were obtained.

2.4. Bioassays

This study encompassed bioassays to test larvicidal activity expressed as LC_{50} in μg/mL against the F_2 *Ae. aegypti* larvae. To

comply with the World Health Organization requirements[26], twenty fourth-instar larvae were placed in glass beakers containing 100 mL of the stock solution (500 μg/mL) for preliminary screening. Plant extracts which produced over 50% larval mortality during the initial testing were serially diluted to 250, 125, and 50 μg/mL in distilled water. The LC_{50} for *Ae. aegypti* larvae was determined in five treatments (including 14 plant parts and four organic solvents) plus two controls dimethylsulphoxide and deionized water (1:100) and 100 mL of distilled water alone. A total of 1 344 larvae per treatment were used, encompassing four replicates of 24 each in a glass beaker containing 100 mL of each plant´s part extract in solvent. Dead larvae in treatments were recorded 24 and 48 h post-exposure and removed daily. Larvae were considered dead if they did not respond to physical stimulus (with a wooden stick). No food was provided to the larvae during the bioassay.

2.5. Statistical analysis

Probit analyses were conducted on *Ae. aegypti* mortality data collected after 24 h and 48 h post-exposure to organic extracts from parts of five plant species using the software IBM-SPSS Statistics V.19 to determine the LC_{50}[27]. A *P*-value of 0.05 or less was considered statistically significant.

3. Results

Bioassays using five species of plants were conducted to determine larvicidal activity against the fourth instar *Ae. aegypti* larvae. Six plant parts and four organic solvents, making a total of 24 combinations, were used to determine LC_{50}. Actual bioassay values of these plant extracts against *Ae. aegypti* larvae were indicated in Table 1. The LC_{50} actual values of the seeds of *A. mexicana* using acetone and hexane as solvents were 60 and 100 μg/mL after 24 h post-exposure and 50 and 80 μg/mL after 48 h, respectively. The hexanoic extract from stem-bark of *P. perniciosum* increased 5-fold of their efficiency ($P < 0.05$) against *Ae. aegypti* from 24 to 48 h post-exposure. The LC_{50} actual value fell from 110 μg/mL to 20 μg/mL, the lowest LC_{50} seen after 48 h post-exposure. Table 2 summarizes relative toxicity of all bioassays performed using four solvents to extract the compounds in aforementioned parts of the plants. When using methanol as solvent, no larvicidal activity was observed from the extracts. The highest larvicidal activity was recorded within 24 h post-exposure when hexane and acetone were used as extracting solvents for the seeds of *A. mexicana*. Similarly, a relatively high activity, but only at 48 h post-exposure, was recorded in roots extract using ethyl acetate. *P. perniciosum*, also showed high larvicidal activity within 24 h post-exposure when the compounds were extracted from stem-bark using hexane. Relative lower larvicidal activities were observed in the extracts of the other three plants (*T. lucida*, *H. excelsa*, and *H. celastroides*).

Table 1

The LC_{50} for *Ae. aegypti* larvae at 24 and 48 h post-exposure to organic extracts from parts of five Mexican plant species.

Plant species	Plant part	Organic solvent	LC_{50} expressed in μg/mL	
			24 h	48 h
T. lucida	Flower	Hexane	250 (220-270)	230 (170-300)
		Ethyl acetate	180 (160-200)	180 (160-210)
		Acetone	570 (490-740)	430 (380-470)
	Leaf	Hexane	260 (130-310)	250 (180-290)
		Ethyl acetate	190 (160-210)	170 (150-190)
	Stem	Hexane	210 (80-250)	210 (120-240)
		Ethyl acetate	180 (120-220)	130 (110-160)
A. mexicana	Seed	Hexane*	100 (100-150)	80 (80-130)
		Ethyl acetate	340 (290-410)	230 (90-290)
		Acetone	60 (40-70)	50 (30-60)
	Flower	Ethyl acetate	390 (250-490)	330 (200-590)
	Leaf	Hexane	400 (220-610)	430 (230-660)
		Ethyl acetate	640 (520-890)	560 (460-860)
		Acetone	300 (300-390)	250 (120-340)
	Stem	Ethyl acetate	340 (90-390)	230 (190-270)
	Root		270 (240-310)	150 (100-190)
		Acetone	230 (160-290)	210 (120-320)
		Methanol	580 (430-730)	530 (450-680)
H. excelsa	Stem	Hexane	610 (510-990)	490 (430-610)
H. celastroides	Leaf	Ethyl acetate	840 (580-930)	500 (380-680)
	Stem	Acetone	740 (520-940)	600 (470-890)
P. perniciosum	Leaf	Hexane	890 (550-990)	190 (140-240)
	Stem-bark		110 (110-130)	20 (10-30)
		Ethyl acetate	580 (430-730)	200 (110-340)

The LC_{50} was estimated by probit analysis. LC_{50} value represents point estimate and LC_{50} values in parentheses represent the standard deviation surrounding point estimate. *: Washed with methanol.

Table 2

Relative toxicity of fourth instar *Ae. aegypti* larvae to organic extracts from parts of five Mexican plant species.

Plant species	Plant part	Solvent							
		Hexane		Ethyl acetate		Acetone		Methanol	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
T. lucida	Flower	+	++	++	++	+	+	-	-
	Leaf	+	++	++	++	-	-	-	-
	Stem	++	++	+	++	-	-	-	-
A. mexicana	Seed	+++	+++	+	++	+++	+++	-	-
	Flower	-	-	+	+	+	+	-	-
	Leaf	+	+	+	+	+	++	-	-
	Stem	-	-	-	-	-	-	-	-
	Root	-	-	++	+++	++	++	+	+
H. excelsa	Stem	-	-	-	-	-	-	-	-
	Leaf	+	+	-	-	-	-	-	-
H. celastroides	Leaf	-	-	+	+	-	-	-	-
	Stem	-	-	-	-	+	+	-	-
P. perniciosum	Leaf	-	-	+	++	-	-	-	-
	Stem bark	+++	+++	+	++	-	-	-	-

+++: LC_{50} < 150 μg/mL; ++: LC_{50} between 150-250 μg/mL; +: LC_{50} > 250 μg/mL; -: No activity detected.

In general, there was no association between the larvicidal activity against *Ae. aegypti* and the polarity of the solvent used in the extract given that a preliminary phytochemical analysis performed with the extracts with the highest activity (Table 3) showed differences in the content of chemical compounds. The presence of alkaloids was overwhelming in the hexanic and ethyl acetate extracts of seeds of *A. mexicana*; however, the cetonic extract did not reveal its presence. Other important components found were sterols and/or terpenoids

and tanins that were identified in all extracts of *A. mexicana*. Meanwhile, in the hexanic extract from stem-bark of *P. perniciosum*, the most abundant chemical groups were cumarins, tannins, sterols/terpenoids, and flavonoids.

Table 3

Preliminary phytochemical analysis performed with the extracts showing the highest larvicidal activity against *Ae. aegypti*.

Compounds	*A. mexicana* (seeds)			*P. perniciosum* (stem-bark)		
	Hexane	Ethyl acetate	Acetone	Hexane	Ethyl acetate	Acetone
Alkaloids	+	-	-	-	-	-
Cumarinas	-	-	-	+	+	+
Tanins	+	+	+	+	+	+
Sterols /terpens	+	+	+	+	+	+
Flavonoids	-	-	-	+	-	-
Glycosides cardoitonics	+	+	+	+	+	+
Sterols	+	+	+	+	+	+
Saponins	-	-	-	-	-	-

-: Not detected; +: Detected.

4. Discussion

Hundreds of plant species have been tested for their toxicities against mosquitoes with a recent review published by Rehman[21,28]. Plant extracts have not yet been used to control dengue vectors in the field, and are not currently under consideration for inclusion into IVMP, but many laboratory trials have been conducted with a view to identify promising candidates.

As expected, there was a variation in the toxicity against the larvae of the mosquitoes because the compounds extracted using different solvents and from different parts of plants tested[29]. In the present study, seeds of *A. mexicana* and stem-bark of *P. perniciosum* using acetone and hexane as solvents showed excellent larvicidal properties (LC_{50} ranged from 60 μg/mL-110 μg/mL) within the first 24 h post-exposure. A range of LC_{50} values between 30.47 μg/mL and 13.58 μg/mL has been observed by Sakthivadivel when using petroleum ether extract from seeds of *A. mexicana* at 24 h[13,19]. Similarly, low LC_{50} values (LC_{50} ranged from 20 μg/mL-50 μg/mL) were observed in our study when using hexane as solvent in both seeds of *A. mexicana* and stem-bark of *P. perniciosum*, respectively at 48 h post-exposure.

It can be pointed out that there was no association between the larvicidal activity and the polarity of the solvent used in the extraction. However, our preliminary phytochemical analysis of the most toxic extracts (*A. mexicana* seeds and *P. perniciosum* stem-bark) against *Ae. aegypti* larvae suggests that the bioactive compounds (alkaloids in *A. mexicana* and coumarins and flavonoids in *P. perniciosum*) present in these two plants are promising candidates for the control of *Ae. aegypti* larvae. Although further evidence is needed, the results of phytochemical analysis showed that the metabolites varied based on the solvent used for the extraction. The toxic effect of *P. perniciosum* stem-bark can be attributed to the presence of coumarins. Previous authors have demonstrated high toxicity of this compound against the larvae of *Ae. aegypti*[30,31]. In addition, flavonoids have also been recognized to have activity as natural insecticide against *Ae. aegypti*[31-33]. Moreover, the major metabolites reported for seeds of *A. mexicana* are berberine and protopine, both toxic alkaloids[31,34].

It would be gratifying to determine the effect of the plant extracts on non-target organisms. These could be done on native aquatic fauna and other biological control of natural enemies or mammals that have access to the water into which the botanical larvicides are to be placed. Such studies of the effect of *A. mexicana* seeds and *P. perniciosum* stem-bark extracts on non-target organisms are currently underway.

In conclusion, two of five species of plants here studied, *A. mexicana* and *P. perniciosum*, may be good candidates to be employed in control programs against the dengue vector, *Ae. aegypti*, as they are highly toxic to the larvae and are abundant as weeds in the Mexican territory and accessible to most inhabitants in the endemic areas[35,36].

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

Dengue fever is an important emerging infectious viral disease transmitted by *Aedes* mosquitoes. The control of this disease relies on the use of chemical insecticides to reduce human-mosquito contact. However, these mosquito vectors are developing resistance to major insecticides used for their control.

Research frontiers

This study showed that *A. mexicana* and *P. perniciosum*, had toxic effect on *Aedes* mosquitoes larvae and may therefore be employed in control programs against these dengue vectors. Due to the availability of these plants in Mexico, their use by the programme to control mosquito larvae may be easy and sustainable.

Related reports

Similar studies in Mexico and elsewhere also demonstrated high toxicity of *A. mexicana* and *P. perniciosum* against the larvae of *Ae. aegypti*. However, such studies were conducted in different settings although they used almost similar experimental designs.

Innovations and breakthroughs

A. mexicana and *P. perniciosum* are known to be toxic against mosquitoes larvae, however, this study demonstrated the toxicity from different parts of the plant using several solvents.

Applications

These plants can be used in the control of mosquito vectors in cheap and sustainable manner. Their availability in Mexico, makes the use by mosquito control programme to be easy. They may further be used to manage insecticide resistance.

Peer review

This is a well written paper which presents valuable information that *A. mexicana* and *P. perniciosum*, have toxic effect on *Aedes* mosquitoes larvae and they may be employed in control programs against these dengue vectors. Their toxicity against the larvae of the mosquitoes varied according to the solvent used.

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