Short Communication

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Larvicidal activity of crude extracts from *Larrea cuneifolia* (Zygophyllaceae) and of its metabolite nordihydroguaiaretic acid against the vector *Culex quinquefasciatus* (Diptera: Culicidae)

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ABSTRACT

Introduction: The aim of the present study was to analyze the larvicidal activity of different crude extracts of *Larrea cuneifolia* and its most abundant lignan, nordihydroguaiaretic acid (NDGA), against *Culex quinquefasciatus.* **Methods:** Chloroform, methanol, and aqueous extracts from *L. cuneifolia* and NDGA were tested against larvae of *Cx. quinquefasciatus* under laboratory conditions. **Results:** The chloroform extract showed the highest larvicidal effect, with an estimated LC_{50} of 0.062 mg/ml. NDGA also demonstrated significant larvicidal activity with an estimated LC_{50} of 0.092 mg/ml. **Conclusions:** These results indicate that the chloroform extract of *L. cuneifolia* and NDGA are promising insecticides of botanical origin that could be useful for controlling *Cx. quinquefasciatus.*

Keywords: *Larrea cuneifolia*.*Culex quinquefasciatus*. larvicidal.

Mosquitoes (Diptera: Culicidae) belong to the most important group of insects in terms of public health, since they are vectors of microorganisms that are pathogenic to humans. Among these, *Culex quinquefasciatus* plays an important role in the transmission of the Saint Louis encephalitis virus (SLEV) and West Nile virus (WNV), 2 arboviruses responsible for human encephalitis, in the United States[1]. In Argentina, SLEV is endemic and its main potential vector seems to be *Cx. quinquefasciatus*. This species of mosquito was found to be infected with SLEV during a human encephalitis outbreak that occurred in 2005 in Córdoba City, Province of Córdoba[2].

Synthetic insecticides have been extensively used for controlling mosquito populations over the past years. However, resistance to pesticides, lethal effects on non-target organisms, and human toxicity are some undesirable effects caused by these methods[3,4]. Thus, the use of natural products represents an alternative pest control method. Plants and their derivatives have been recognized as an important natural resource of insecticides[4]. Extracts from plants constitute a rich source of bioactive compounds that are biodegradable and potentially suitable for use to control mosquitoes[5-8].

Ethnobotanical studies point out that different species of Argentinean autochthonous plants are used for medicinal purposes[9]. Among these, those belonging to genus *Larrea* (Zygophyllaceae) are most remarkable. This genus is widely distributed in the New World with 5 recognized species: *Larrea nitida, L. ameghinoi, L. divaricata,* and *L. cuneifolia*, which grow in South America, and *L. tridentata*, which grows exclusively in North America. *L. cuneifolia* grows mainly in the central part of Argentina[10]. An interesting characteristic of the genus *Larrea* is the large number of chemical compounds found in the resin of the leaves and stems of these plants, especially their high content of lignans, flavonoids, and saponins. These compounds have a wide spectrum of bioactivity, with nordihydroguaiaretic acid (NDGA) being the most abundant lignan in this genus[11]. Antitumor, antioxidant, antiseptic, anti-pyretic, and antifungal effects are some of the bioactivities attributed to the *Larrea* genus and NDGA[11].

Different members of the Zygophyllaceae family have demonstrated larvicidal activity against *Cx. pipiens*[6]. Likewise, extracts of *L. divaricata* have demonstrated antifeeding and repellent activity against *Sitophilus oryzae* (Coleóptera: Curculionidae)[12]. However, research on the larvicidal activity of *Larrea* species against mosquitoes is scarce. Therefore, the aim of this study was to evaluate the larvicidal effects of different crude extracts of *L. cuneifolia* and of NDGA against *Cx. quinquefasciatus.*

The aerial parts of the species *L. cuneifolia* were collected from Santa María de Punilla, Province of Córdoba, Argentine. The plant material was identified by Dr. Gloria Barboza (Instituto Multidisciplinario de Biología Vegetal-Consejo Nacional de Investigaciones Científicas y Técnicas-Universidad

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Nacional de Córdoba), and a voucher specimen was deposited in the Botanical Museum of Córdoba as MSO-33.

From 800g of dried and fragmented vegetal material, 2 extracts were obtained in a Soxhlet extractor, using chloroform (Cl_3CH) and methanol (MeOH), respectively, as solvents. On the other hand, 430g of dried and fragmented plants were extracted with water at 85ºC and 25°C for 1 h and subsequently filtered, in order to obtain hot (HAq) and cold (CAq) aqueous extracts, respectively. The organic extracts were concentrated *in vacuo*, and the aqueous extracts were lyophilized, yielding the following dried extracts: Cl_3CH extract, 10.16 g (1.3%); MeOH extract, 16.64 g (2.1%); HAq extract, 21.18 g (4.9%); and CAq extract, 28.92 g (6.7%). NDGA from *L. divaricata* (Zygophyllaceae) was purchased from Sigma-Aldrich (USA). NDGA and the chloroform and methanol extracts were dissolved in dimethyl sulfoxide (DMSO); the aqueous extracts were dissolved in distilled water.

Cx. quinquefasciatus larvae were collected from Córdoba City and then colonized and maintained continuously for several generations under laboratory conditions. The breeding methodology for mass rearing followed the general guidelines of Gerber et al[12]. Larvae were reared in 1,000-ml plastic trays of and fed powdered liver (0.25 mg/larvae/day). Mass rearing was carried out at 25 ± 2°C with a 13:11 (light:dark) photoperiod. Larvae from these colonies were used for the assays.

Three concentrations of each previously dissolved plant extract (0.5, 0.25, and 0.1 mg/ml) and 4 concentrations of NDGA (0.2, 0.1, 0.05, and 0.025 mg/ml) were tested. The extracts and NDGA were placed in plastic trays containing 30 third stadium larvae in 100 ml of distilled water; these were kept at 25 ± 2ºC with a 13:11 (L:D) photoperiod. The larvae were fed daily with liver powder (0.25 mg per larva per day). Four concentrations of DMSO (0.5, 0.25, and 0.1 %v/v for the extract assays and 0.2 %v/v for the NDGA assay) and distilled water were used as controls. The larvae were exposed to these solutions and mortality was registered every 24 h over a period of 72 h. Five replications of each treatment were performed.

To evaluate the larvicidal effects of the different extracts and NDGA, the percentages of dead larvae were compared by Kruskal–Wallis test. Larvae were considered dead when they did not respond to stimulus or when they did not rise to the surface of the solution. LC_{50} and LC_{90} values were calculated by Probit regression (SPSS 17.0).

Larval mortality rates produced by each extract, expressed as percentages, are shown in **Figure 1**. Analysis of Variance tests comparing percentages of mortality between the treatments and controls showed significant differences, depending on the concentrations and extracts used (Kruskal-Wallis; H = 69.31; p<0.0001). The Cl_3CH extract showed the greatest larvicidal effect (84.67–100%) compared to other extracts, with no significant differences between the 2 higher concentrations assayed (p > 0.05). With the MeOH extract, the greatest mortality was observed at 0.5 mg/ml (83.3%) and was not significantly different from the mortality obtained with the Cl_3CH extract at 0.1 mg/ml (p > 0.05). The aqueous extracts did not show larvicidal activity, yielding values lower than 30% (**Figure 1**).

Significant differences were detected between the different concentrations of NDGA tested (Kruskal-Wallis; H = 14.06; p = 0.0138). The highest values of mortality observed were 100 and 68.89% at the 0.2 and 0.1 mg/ml concentrations, respectively (**Figure 2**). The mortality rates registered with the 2 remaining concentrations were low (28.9 and 33.3%, respectively). Mortality rates in the DMSO controls were low (< 20%), and there was a complete absence of larval mortality in the water controls (**Figures 1** and **2**).

The values of the different extracts and NDGA, estimated by the LC_{50}–LC_{90}, regression equation, and chi square methods, are presented in **Table 1**. The chloroform extract showed the lowest LC_{50}–LC_{90} values (0.062–0.130 mg/ml), making it the most toxic extract against *Cx. quinquefasciatus*.

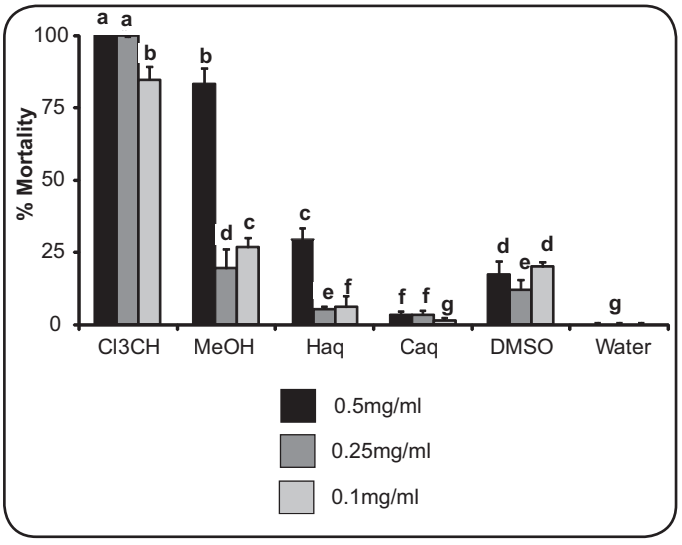

FIGURE 1 - Percentage larval mortality of* Culex quinquefasciatus *after exposure to different concentrations of* Larrea cuneifolia *extracts and their respective dimethyl sulfoxide (DMSO) and water controls. Different letters between columns indicate significant differences (p < 0.05) between treatments and controls based on a Kruskal-Wallis test on ranked larval mortality.

Cl_3CH: chloroform extract; **MeOH:** methanol extract; **HAq:** hot aqueous extract; **CAq:** cold aqueous extract; **DMSO**: dimethyl sulfoxide.

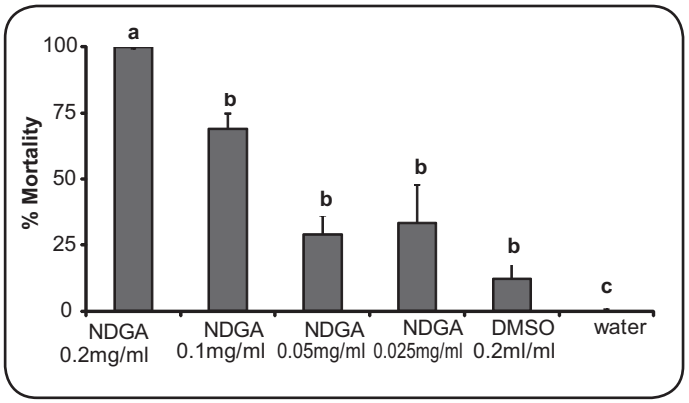

***FIGURE 2 - Percentage larval mortality of* Culex quinquefasciatus *after exposure to different concentrations of NDGA and their respective DMSO and water controls. Different letters between columns indicate significant differences (p < 0.05) between treatments and controls based on a Kruskal-Wallis test on ranked larval mortality. NDGA:** nordihydroguaiaretic acid; **DMSO**: dimethyl sulfoxide.*

TABLE 1 - Lethal concentrations (mg/ml) of different extracts from **Larrea cuneifolia** *and nordihydroguaiaretic acid on third-instar larvae of* **Culex quinquefasciatus.**

Extract	LC_{50} (95% CL), mg/ml	LC_{90} (95% CL), mg/ml	Regression equation	Chi square
Cl_3CH	0.062 (0.044–0.080)	0.130 (0.107–0.172)	$y = 18.814x–1.166$	50.179
MeOH	0.335 (0.278–0.409)	0.588 (0.494–0.760)	$y = 5.05x–1.69$	81.880
NDGA	0.092 (0.069–0.127)	0.172 (0.134–0.260)	$y = 16.119x–1.484$	71.798

Lethal concentrations (mg/ml) of different extracts from *Larrea cuneifolia* and NDGA on third-instar larvae of *Culex quinquefasciatus.* **LC_{50}:** lethal concentration that causes 50% mortality; **LC_{90}:** lethal concentration that causes 90% mortality; **CL:** confidence limits; **Cl_3CH**: chloroform extract; **MeOH**: methanol extract; **NDGA:** nordihydroguaiaretic acid.

The results in this study revealed the larvicidal effects of the Cl_3CH and MeOH extracts of *L. cuneifolia* against *Cx. quinquefasciatus,* with LC_{50} values of 0.062 and 0.335 mg/ml, respectively, showing high larvicidal activity compared to other plant species present in Argentina. Ethanolic extracts from *Melia azedarach*, also a common and widely distributed species in Argentina, showed larvicidal activity against *Aedes aegypti,* with LC_{50} values ranging from 0.76 to 11.5 mg/ml[7,8]. The larvicidal activity data of *L. cuneifolia* reported in the present study are comparable to those achieved by Batallán et al[5], who demonstrated the larvicidal effect of the Cl_3CH extract from *L. cuneifolia* on the larvae of *Ae. aegypti*, with LC_{50} and LC_{90} values of 0.069 and 0.11 mg/ml respectively.

Cabral et al.[14] demonstrated that NDGA had inhibitory effects on the ecdysis of *Rhodnius prolixus* (Hemiptera: Reduviidae). On the other hand, it has also been demonstrated that NDGA extends the life cycle of *Ae. aegypti* at concentrations ranging between 0 and 0.1 mg/ml[15]; nevertheless, there is no evidence of pesticide activity for this metabolite. In this study, NDGA revealed an important larvicidal effect at the highest concentration. However, the LC_{50} estimated for NDGA was higher than the value estimated for the Cl_3CH extract. The presence of NDGA in the Cl_3CH, MeOH, and HAq extracts from *L. divaricata* was detected and quantified by high-performance liquid chromatography. The highest amount of NDGA was detected in the Cl_3CH and HAq extracts (B. Konigheim, submitted for publication). The larvicidal activity of the organic extracts in this study was higher than those of the aqueous extracts. These results suggest that the toxicity of the Cl_3CH and MeOH extracts is not exclusively due to NDGA, but to a combination of NDGA and other metabolites present in the extracts.

In conclusion, the present study indicates that the Cl_3CH extract of *L. cuneifolia* is a promising tool for controlling *Cx. quinquefasciatus.* These preliminary results regarding the larvicidal activity of extracts of *L. cuneifolia* are of great interest, since they set the precedent for this activity, which has not been reported thus far. In addition, the larvicidal activity observed with NDGA was remarkable, which leads to further investigations into the possible mechanisms of action of this metabolite and the identification of additional compounds in *L. cuneifolia.* These results are particularly interesting because *L. cuneifolia* is a native species widely distributed in central Argentina. Field studies should be performed to confirm the results and assess the feasibility of their use in controlling insects of medical concern.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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