Determination of the Insecticidal Activity of *Heliopsis longipes* A. Gray Blake, an Endemic Plant of the State of Guanajuato

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Summary
Mosquitoes are involved in transmission of infectious diseases like malaria which affect human health, causing economic losses due to expensive treatments and job incapacity of patients. Strategies to minimize transmission of this disease are the employ of chemical insecticides that are excellent methods to reduce insect populations; however it causes deleterious effects on human health and environmental damage. Therefore is necessary to explore harmless alternatives, such as plant extracts which are potential source of natural insecticides. In this work we evaluated insecticidal properties of Heliopsis longipes A. Gray Blake against third instar larvae of Anopheles albimanus, malaria vector. Results showed that H. longipes A. Gray Blake has insecticide properties to control insect involved in malaria transmission.
Key words: Heliopsis longipes A. Gray Blake, Alcamides, Natural insecticides.

Introduction
Mosquitoes pose a public health problem because they act as vectors for transmitting infectious diseases such as malaria, dengue, and filariasis. They are also pests that cause allergic reactions like local skin responses and systemic reactions such as angioedema and urticaria in humans (Sen-Sung et al., 2003). In Mexico, female mosquitoes of Anopheles albimanus, A. pseudopunctipennis, A. quadrimaculatus, and A. aztecus are the primary malaria vectors. These mosquitoes thrive in regions with abundant vegetation, swamps, flooded areas, and marshy valleys, among other environments favorable for the vector's development, where eradication of the disease

has been difficult (Velázquez-Monroy et al., 2003). Public health institutions employ strategies for malaria control through the use of chemical insecticides, including organophosphates and pyrethroids. Although these substances reduce the insect-vector population, they pose potential health risks to humans and create severe environmental issues by affecting biodiversity (Anonymous, 2008). Moreover, continuous use of these products has led to the development of resistance in mosquitoes, making vector control more difficult and leading to disease recurrence. For these reasons, it is essential to explore alternative methods to control insect populations while minimizing environmental impacts (Hay et al., 2002; Maharaj et al., 2011).

Plants offer a promising alternative in the search for more effective, cost-efficient, and environmentally friendly insecticides. Plants are a significant source of bioactive compounds, some of which have been shown to act as insecticides against mosquito larvae and adults, or as insectistatics, inhibiting the normal development of insects and thus reducing vector populations. Among the plants with insecticidal properties, it has been shown that pyrethrum, extracted from the flowers of Chrysanthemum cinerariaefolium, is effective in controlling pest insects (Casida, 1980). The neem tree (Azadarachta indica), used to treat a wide range of human and livestock diseases, has mosquito-killing properties and has been employed to eradicate insect vectors of various diseases (Shaalan et al., 2005). Furthermore, species of the genus Tagetes have been found to be effective insecticides against both the larval and adult stages of mosquitoes. Eclipta paniculata demonstrates larvicidal activity, while Polyalthia longifolia exhibits both larvicidal and mosquito growth inhibitory effects (Mittal et al., 2003).

In Mexico, it is estimated that there are 300,000 plant species, making it one of the countries with the greatest floral diversity in the world and one with a strong tradition of using medicinal plants to treat various ailments (Toledo, 1994). Within this plant diversity, extracts from plants such as Ricinus communis (castor bean), Datura candida (angel's trumpet), Satureja laevigata (poleo), Rosmarinus officinales (rosemary), and Ruta graveolens (rue) have been shown to control pest insects in family gardens, particularly for tomato, squash, and bean crops (Vázquez, 2005).

In the state of Guanajuato, Heliopsis longipes A. Gray Blake is a plant species whose roots are traditionally used as a vermifuge, food flavoring, local anesthetic for toothache, treatment for mouth ulcers, athlete's foot, and many other traditional uses in various municipalities of the Sierra Gorda region. This plant, commonly known as Chilcuague or herb of the tooth, accumulates a compound called affinin in its roots. Affinin (N-isobutyl-2E,6Z,8E-decatrienamide) belongs to the group of olefinic alkamides and makes up to 90% of the total metabolites in the roots. It is responsible for the biological effects attributed to the plant. Like pyrethrum, Heliopsis longipes root extracts exhibit paralyzing action and toxicity against insects, including the housefly (Crombie and Krasinski, 1962), the lepidopteran Diaphania hyalinata, and the dipteran Aedes aegypti, a vector of dengue (Jacobson, 1971). Due to these properties, the plant was used as an insecticide during World War II (Little, 1948). Studies on related species have shown that scabrine, isolated from Heliopsis scabra Dunal, is an even more potent

insecticide than pyrethrins, though its use was discontinued due to its toxicity to mammals (Roark, 1951).

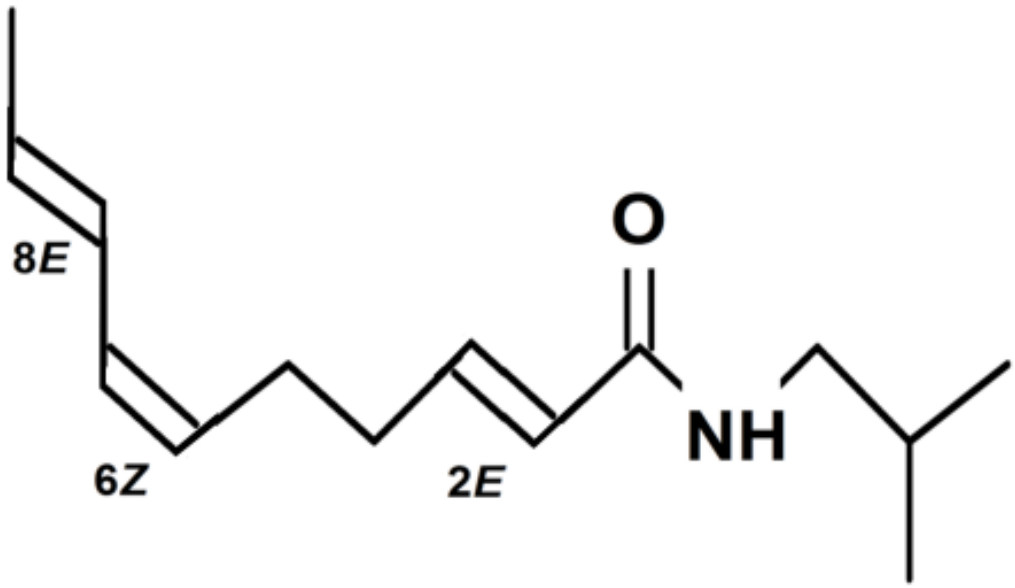

Figure 1: Structural formula of affinin, N-isobutyl-2E, 6Z, 8E-decatrienamide.
The main bioactive component of the ethanolic extract of H. longipes A. Gray Blake.
Molecular formula: $C_{14}H_{13}NO$
Molecular weight: 221 g/mol.

In recent years, resistance to insecticides and climate change have contributed to the resurgence of malaria. Additionally, no vaccine is currently available for this disease. Therefore, it is necessary to explore alternatives to control populations of vectors that transmit infectious diseases. Based on this, the present study determined the insecticidal potential of H. longipes A. Gray Blake against larvae of A. albimanus, the primary vector of malaria in Mexico.

Materials and Methods

Collection of Plant Material

Roots of H. longipes A. Gray Blake (Figure 2) were collected in the municipality of Puerto de Tablas, Xichú, in the Sierra Gorda region of the state of Guanajuato, at altitudes of approximately 2,589 meters above sea level, in disturbed oak forests (Quercus sp.) on steep slopes. The authenticity of the plant material was confirmed by Dr. Jerzy Rzedowski from the Institute of Ecology in Pátzcuaro, Michoacán, where reference specimens were deposited (García-Chávez et al., 2004).

Figure 2: Roots of H. longipes A. Gray Blake.

Extraction Process

The ethanolic extract of H. longipes A. Gray Blake roots was obtained from 1.5 kg of dried roots, which were pulverized and macerated with 10 L of absolute ethanol for one week at room temperature. After this period, the extract was filtered using Whatman No. 2 filter paper to remove suspended particles. The resulting extract was concentrated to approximately 1 L using a Büchi 461 rotary evaporator at 50°C. The extract was then stored at 4°C until use.

Analysis of the H. longipes A. Gray Blake Extract

Samples of the ethanolic extract were analyzed using a Gas Chromatograph (GC Hewlett-Packard model 5890) equipped with an HP-1MS capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) coupled to a Mass Spectrometer (Hewlett-Packard model 5972 MSD). The instrument was programmed with the following operating conditions: the injector temperature was maintained at 200°C, the initial oven temperature was set at 150°C for 3 minutes, with an increase of 4°C per minute until reaching a final temperature of 300°C, which was held for 20 minutes. Helium was used as the carrier gas at a constant flow of 1 mL/min. The extract was quantified based on a calibration curve of affinin previously prepared in the laboratory.

Anopheles albimanus Colony
The mosquito colony of A. albimanus was established in the Insectary of Cinvestav, Irapuato Unit, using eggs donated by Dr. Humberto Lanz Mendoza from the National Institute of Public Health in Cuernavaca, Morelos. The eggs were hatched in trays with chlorine-free tap water and incubated at 28°C, 75% relative humidity, and a 12-hour light:12-hour dark cycle.

Larvicidal Assay Against Anopheles albimanus
The assay followed the procedure recommended by the World Health Organization (WHO). In plastic containers holding 50 ml of chlorine-free tap water, 10 third-instar larvae of A. albimanus were placed. Different concentrations of the ethanolic extract (0–200 ppm) were then added. Commercial insecticide Abate® and absolute ethanol were used as positive and negative controls, respectively. Each treatment was performed in triplicate. The treatments, along with the controls, were incubated under the same conditions as the mosquito colony. The larvicidal effect was determined after 48 hours, with positive results indicated by dead larvae or those showing no movement. Median lethal dose (LD50) values were calculated using Probit software (Raymond, 1985).

Results and Discussion

Affinin is the major compound in the ethanolic extract of H. longipes A. Gray Blake root. To obtain the extract, two rounds of maceration with 96% ethanol were performed to maximize the extraction of compounds present in the roots of H. longipes A. Gray Blake, yielding approximately 10 L of ethanolic extract. A sample of the extract was concentrated under reduced pressure to remove the solvent, resulting in a yellow oil, indicative of the lipid nature of the alkamides. To determine its composition, the oil was dissolved in absolute ethanol and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), which identified the compounds present in the roots of H. longipes A. Gray Blake.

The chromatographic analysis revealed a major compound eluting at a retention time of 11.53 minutes (Figure 3), with a mass spectrometry (MS) fragmentation pattern of m/z (mass/charge) = 221 (10), 192 (4), 141 (100), 126 (39), 98 (26), 81 (94), 68 (14), 53 (12). When compared with the NIST library, the major compound in the roots of H. longipes A. Gray Blake was confirmed to be affinin (N-isobutyl-2E,6Z,8E-decatrienamide), with m/z peaks at 221 and 141 corresponding to the molecular ion and the parent ion, respectively (Figure 4).

Additionally, the chromatogram revealed minor compounds in the extract (Figure 3). Based on their MS fragmentation patterns compared with the database, these were identified as various alkamides: (b) N-(2-methylbutyl)-2E,6Z,8E-decatrienamide, (c) N-isobutyl-2Z-monoen-8,10-diyn-undecamide, (d) N-isobutyl-2E-monoen-8,10-diyn-undecamide, and (e) 2E,6Z,8E-decatrienoate of bornyl (Table 1), indicating that the ethanolic extract is composed of a mixture of alkamides.

Finally, the GC-MS analysis determined the concentration of the extract, estimated to be 142.2 mg/mL, based on the major alkamide, affinin, which was used as an indicator of the extraction process's efficiency.

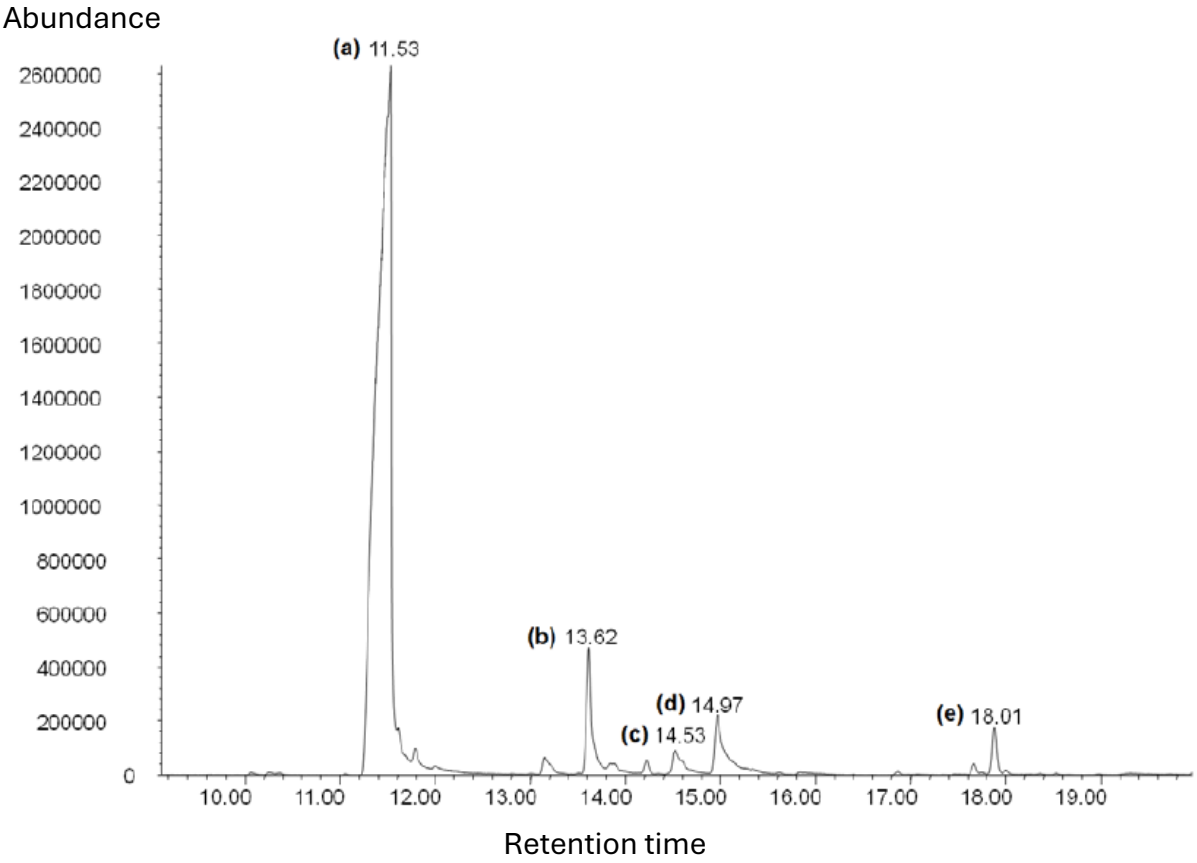

Figure 3: Composition of the ethanolic extract from the roots of H. longipes A. Gray Blake. Chromatogram obtained by GC-MS of the ethanolic extract from the roots of H. longipes A. Gray Blake. The highest peak corresponds to (a) N-isobutyl-2E,6Z,8E-decatrienamide, the bioactive compound present in the plant's roots. The remaining peaks correspond to various alkamides present in the extract: (b) N-(2-methylbutyl)-2E,6Z,8E-decatrienamide, (c) N-isobutyl-2Z-monoen-8,10-diyn-undecamide, (d) N-isobutyl-2E-monoen-8,10-diyn-undecamide, and (e) 2E,6Z,8E-decatrienoate of bornyl.

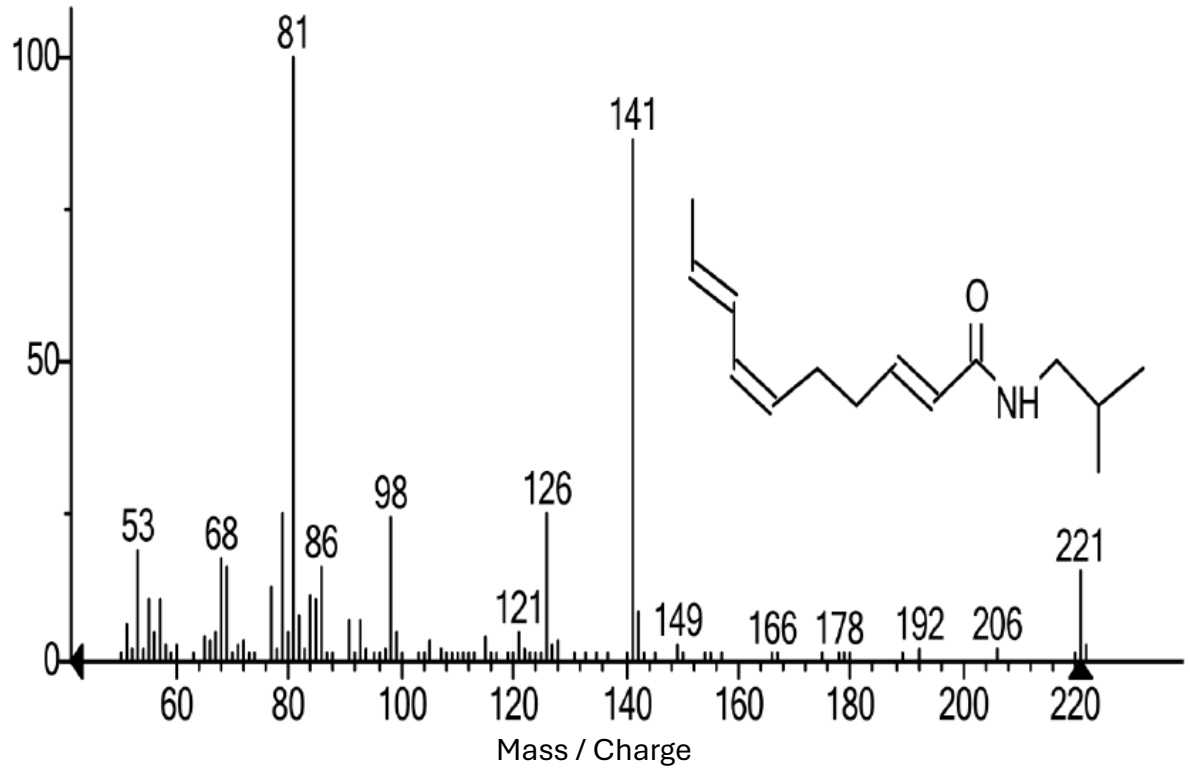

Figure 4: Fragmentation pattern by Mass Spectrometry of affinin, N-isobutyl-2E, 6Z, 8E-decatrienamide.

When comparing the LD50 of the H. longipes A. Gray Blake extract with the positive control, the commercial insecticide Abate® (Table 2), it was found that a larger quantity of the ethanolic extract is required to achieve the same result. However, it is important to highlight that mosquitoes in the environment have developed resistance to the insecticides used in prevention campaigns, including Abate®. As a result, increasingly larger quantities of the insecticide have been required, posing a serious threat to human health and ecosystem biodiversity. Given this scenario, the use of the H. longipes extract as an insecticide could be an excellent alternative for controlling resistant mosquito populations, contributing to a reduction in the incidence of vector-borne diseases. This presents an ecological alternative, as it reduces the spraying of chemicals into the environment.

Table 1. Alkamides present in the ethanolic extract of the roots of H. longipes A. Gray Blake.

Compuesto	TR	EM (*m/z*)
(a) *N*-isobutil-2*E*,6*Z*,8*E* decatrienamida	11.53	221 (10), 192 (4), 141 (100), 126 (39), 98(26), 81 (94), 68 (14), 53 (12)
(b) *N*-(2-metilbutil)-2*E*,6*Z*,8*E* decatrienamida	13.62	235 (12), 53 (18), 69 (18), 81 (100), 86 (30), 98 (14), 126 (12), 155 (88)
(c) *N*-isobutil-2*Z*-monoen-8,10 diin-undecamida	14.53	230 (1), 146 (10), 141 (9), 131 (73), 117 (76), 103 (27), 91 (95), 57 (100)
(d) *N*-isobutil-2*E*-monoen-8,10 diin-undecamida	14.97	231 (17), 57 (45), 63(35), 79 (21), 91 (100), 103 (41), 116 (45), 131 (58)
(e) 2*E*,6*Z*,8*E* decatrienoato de bornilo	18.01	302 (0.2), 137 (76), 121 (9), 109 (13), 93 (14), 81 (100), 69 (7), 55 (6.1)

TR = Retention Time; EM = Fragmentation pattern by mass spectrometry.

Table 2. Median Lethal Dose (LD50) of the ethanolic extract of H. longipes A. Gray Blake against Anopheles albimanus.

Compound	**DL_{50} ppm**	**95 % IC**
ethanolic extract	2.85	1.98 – 4.09
Reduce	< 1	ND

Mortality was determined 48 hours post-exposure to the evaluated compounds. SD = Standard deviation; CI = 95% Confidence Interval; ND = Not determined

Conclusions

The root extract of H. longipes A. Gray Blake represents a potential source of insecticides that can be used as an alternative to control mosquito populations that transmit malaria. Its advantage lies in being composed of a mixture of alkamides that may exert a synergistic effect, making it unnecessary to carry out purification processes for the alkamides.

References

Anonymous. 2008. Methods for controlling Aedes aegypti, the mosquito vector of the dengue virus in Mexico. Vector-borne disease control program [www.cenave.gob.mx/dengue/insecticida.pdf].

Casida J. 1980. Pyrethrum flowers and pyrethroid insecticides. Environmental Health Perspectives 34:189-202.

Crombie L and Krasinski. 1962. Synthesis of N-isobutyldeca-trans-2,cis-6,trans-2,cis-6,cis-8 trienamide. Chemical Industry 983-984.

García-Chávez A, Ramírez-Chávez E, Molina-Torres J. 2004. The genus Heliopsis (Heliantheae, Asteraceae) in Mexico and the alkamides present in its roots. Acta Botánica Mexicana 69:115-131.

Hay S I, Cox J, Rogers D J, Randolph S E, Stern D I, Shanks G D, Myers M F, Snow R W. 2002. Climate change and the resurgence of malaria in the East African highlands. Nature 415:905-909.

Jacobson M. 1971. The unsaturated isobutylamides. In: Naturally occurring insecticides. Jacobson M and Crosby D G (eds). Marcel Dekker, New York, pp. 137-173.

Little E. 1948. The chilcuague (Heliopsis longipes). Insecticidal plant. Boletín de la Sociedad Botánica de México 7:23-27.

Maharaj R, Maharaj V, Crouch N R, Bhagwandin N, Folb P I, Pillay P, Gayaram R. 2011. Screening for adulticidal bioactivity of South African plants against Anopheles arabiensis. Malaria Journal 10:223.

Martínez M. 1994. Medicinal plants of Mexico. Ediciones Botas 6th ed., pp. 113-115.

Mittal P K, Subbarao S K. 2003. Prospects of using herbal products in mosquito vector control. ICMR Bulletin 33:1-10.

Molina-Torres J, García-Chávez A, Ramírez-Chávez E. 1999. Antimicrobial properties of alkamides present in flavoring plants traditionally used in Mesoamerica: Affinin and capsaicin. Journal of Ethnopharmacology 64:241-248.

Raymond M. 1985. Presentation of a Basic program for log-probit analysis for microcomputers. Cahiers ORSTOM Series Entomology Medicine Parasitology 23:117-121.
Roark R C. 1951. Scabrin. Soaps and Sanitary Chemicals 27:125-137.
Sen-Sung C, Hui-Ting C, Shang-Tzen C, Kun-Hsien T, Wei-June Ch. 2003. Bioactivity of selected plant essential oils against Aedes aegypti larvae, the yellow fever mosquito. Bioresource Technology 89:99-102.
Shaalan E A, Canyon D, Younes M W F, Abdel-Wahab H, Mansour A H. 2005. A review of botanical phytochemicals with mosquitocidal potential. Environment International 31:1149-1166.
Toledo V M. 1994. The biological diversity of Mexico. New challenges for research in the 1990s. Ciencias UNAM pp. 43.
Vázquez R F. 2005. Evaluation of plant extracts in controlling pest insects at the family garden level. Residence Report. Oaxaca Agricultural Technology Institute.
Velázquez-Monroy O J, Gutiérrez-Trujillo G, Dávila-Torres J, Cabral-Soto J, Heredia-Jarero N M. 2003. Epidemiology of malaria. Epidemiology 20(37):23.
Ximénez F. 1615. Qvatro libros de la naturaleza. Mexico, Diego López Dávalos, pp. 224.

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