The genus Heliopsis (Heliantheae; Asteraceae) in Mexico and the alkamides present in its roots.

ABRAHAM GARCÍA-CHÁVEZ, ENRIQUE RAMÍREZ CHÁVEZ and JORGE MOLINA-TORRES

Phytobiochemistry Laboratory, Department of Biotechnology and Biochemistry, CINVESTAV-IPN Irapuato Unit, km 9.6 Libramiento Norte, P.O. Box 629, 36500 Irapuato, Guanajuato, Mexico

Abstract

The genus Heliopsis belongs to the tribe Heliantheae in the Asteraceae family. It includes 14 species, most of them endemic to Mexico. One of the species, Heliopsis longipes, is utilized in traditional medicine. The high level of alkamides in its roots has already been chemically characterized. The study of these metabolites is displayed in a comparative analysis on the presence of alkamides in six Mexican species of Heliopsis. The quantitative and qualitative analysis of the alkamides stored in roots of each species is presented. Chemotaxonomic importance of alkamides in this genus is also discussed. In this work we review and update the bibliography referring to the taxonomy of the genus.

Key words: alkamides, Asteraceae, Heliantheae, Heliopsis, Mexico.

Botanical Aspects

General Information Related to Heliopsis
The genus Heliopsis belongs to the tribe Heliantheae of the family Asteraceae.
This tribe includes around 2,500 species grouped into 189 genera and is divided into ten subtribes. Heliopsis, along with Zinnia, Philactis, Sanvitalia, Acmella, Podachaenium, Squamopappus, Spilanthes, and Salmea, forms the subtribe Zinniinae (Bremer, 1994). Members of this subtribe share morphological features such as solitary heads, conical or hemispherical receptacles, fertile and persistent ligulate flowers, fertile disc flowers, and laterally compressed or angular disc achenes.

The main difference that separates Heliopsis species from other genera is the following combination of characteristics: the presence of fertile disc flowers, persistent and fertile ligulate flowers, sessile and persistent corollas, thick achenes with 3 to 4 angles, and the absence of a pappus.

Although restricted to the Western Hemisphere, the distribution of this genus is broad, ranging from the Great Plains and eastern United States to Bolivia. The habitats where its species thrive are varied, from arid regions to humid areas, and from warm climates to temperate ones. The abundance of some species is enhanced by disturbance, and at least one species behaves as a weed. The genus is represented by 14 known species, although this number varies depending on the criteria of different authors. Of these species, ten are found in Mexico, and eight of them are endemic (Table 1).

Taxonomic studies on Heliopsis have been scarce, which has caused uncertainty in the interpretation of some of its taxa. These studies have been based on morphological characteristics. Species of this genus are perennial or annual herbaceous plants; they have opposite or sometimes alternate leaves, which range from subentire to toothed; terminal or axillary heads; hemispherical to broadly campanulate involucres, with subequal-sized bracts, the outer ones often herbaceous; convex to conical receptacles with persistent chaff; fertile ligulate flowers with sessile (tube-less) and persistent corollas, which may be yellow, red, or purple; hermaphroditic disc flowers, although often largely sterile, with tubular corollas that are yellow, yellow-brown, or purple; anthers with slightly arrow-shaped bases; linear, flattened style branches in hermaphroditic flowers, with short appendages; thick achenes with 3 to 4 angles, and a pappus absent (Rzedowski, Rzedowski et al., 2001).

Most representatives of Heliopsis are perennial plants, with the exception of five annual species (H. annua, H. anomala, H. filifolia, H. parviceps, and H. sinaloensis), which are endemic to Mexico. The perennial species have thick, fleshy roots that may be divided or undivided.

Table 1: Known Species of Heliopsis

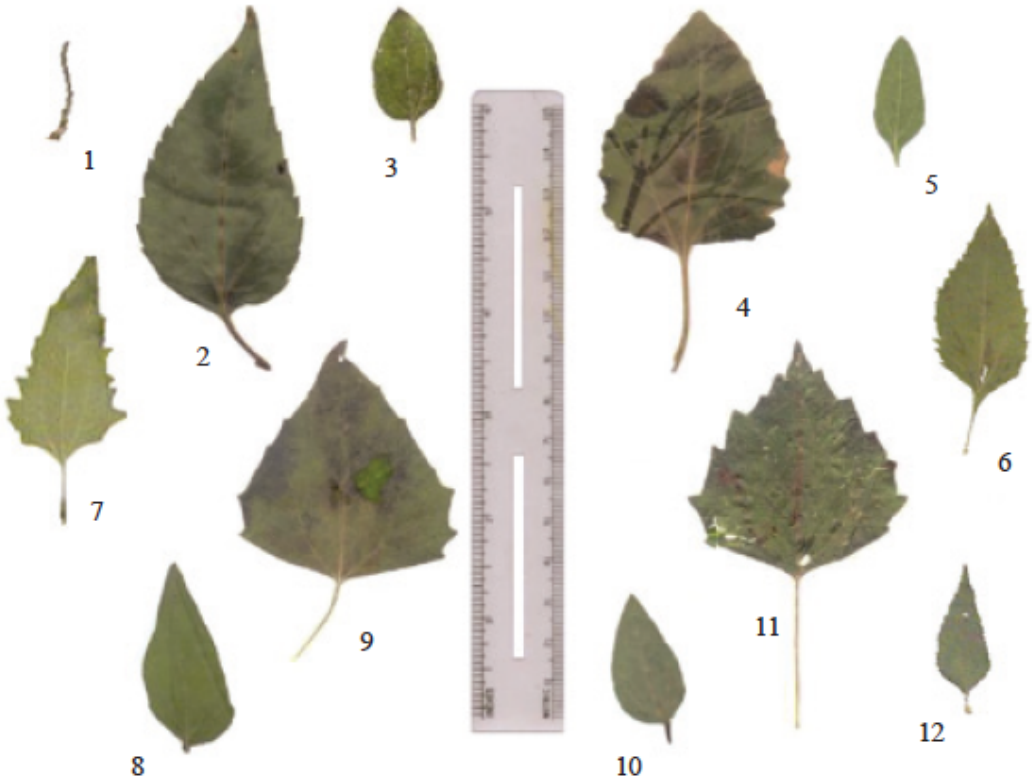
Species	Geographical Distribution
Of restricted distribution	Located in a small geographical area, they are bounded by isolated orographic systems, such as small mountain ranges, plateaus, or isolated valleys, and even endorheic basins.
H. filifolia S. Wats.	Cuatro Ciénegas, Carneros, and Puerto Colorado, Coahuila
H. longipes (Gray) Blake	Sierra Gorda, in the central part of the country, in the bordering region of the states of Guanajuato, San Luis Potosí, and Querétaro
H. sinaloensis B. L. Turner	Imala, Sinaloa
Of regional distribution	Located across several states in the country, but without exceeding national boundaries
H. annua Hemsl.	Mexican Plateau, mainly in the Bajío region. Jalisco, Guanajuato, Querétaro, Michoacán, State of Mexico, Mexico City, Morelos, Tlaxcala, Puebla, Hidalgo, San Luis Potosí, Zacatecas, Aguascalientes, Durango, Chihuahua, and Coahuila
H. anomala (M. E. Jones) B. L. Turner	Region of coastal mountains and canyons of the Gulf of California (Baja

	California, Baja California Sur, and Sonora)
H. novogaliciana B. L. Turner	Sierra Madre Occidental. Jalisco, Nayarit, Sinaloa, Chihuahua, and Durango
H. parviceps Blake	Pacific slope of Mexico. Sinaloa, Michoacán, Guerrero, State of Mexico
H. procumbens Hemsl.	Trans-Mexican Volcanic Belt. Jalisco, Michoacán, State of Mexico, Mexico City, Hidalgo, Morelos, Tlaxcala, Puebla, and Veracruz

Of wide distribution	The distribution of these species exceeds national boundaries
H. buphthalmoides (Jacq.) Dunal	The species with the widest geographical distribution in the genus. Sierra Madre Occidental, Sierra Madre del Sur, Central American Cordillera, and the Andes. Found in Durango, Sinaloa, Nayarit, Jalisco, Michoacán, State of Mexico, Morelos, Guerrero, Puebla, Oaxaca, and Chiapas in Mexico; throughout Central America, Colombia, Venezuela, Peru, and Bolivia in South America.
H. parviflora Gray	Arid and semi-arid zones of northern Mexico and the southern United States. Found in Zacatecas, Aguascalientes, Durango, Nuevo León, Coahuila, Chihuahua, and Sonora in Mexico; California, Arizona, New Mexico, and Texas in the United States.

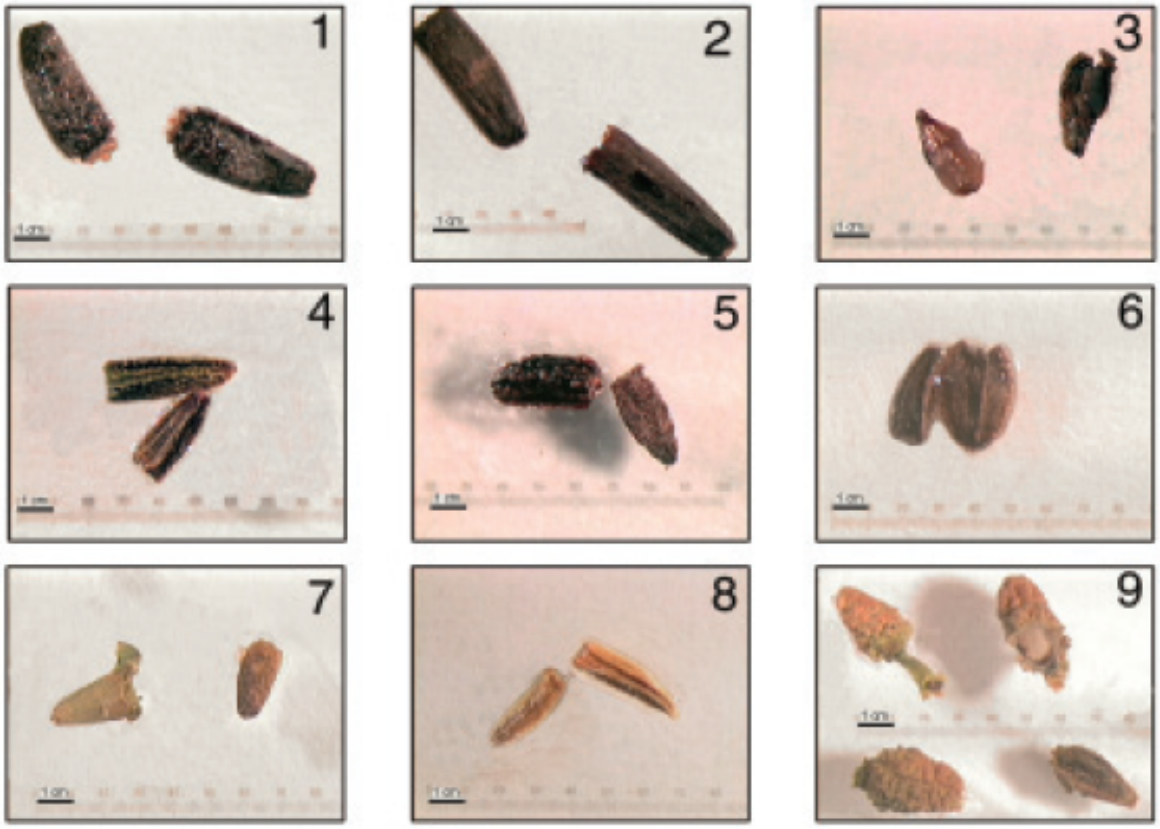
Outside of Mexico	Distributed only outside of Mexico
H. decumbens Blake	Cajamarca, Peru. Known only from the type locality
H. gracilis (Gray) Nutt.	From southwestern Georgia, northern and central Florida to southern Alabama
H. helianthoides (L.) Sweet	Eastern United States
H. lanceolata Blake	Santander, Colombia. Known only from the type locality

In Picture 1, it can be observed that the leaves of Heliopsis have deltoid, lanceolate, ovate, or lance-ovate shapes, with the filiform shape being specific to H. filifolia. The leaf margin is an important characteristic. Most species have toothed or semi-toothed leaves, though some have nearly entire or irregularly crenate margins. The texture is generally firm, except for annual plants, which usually have thin and fragile leaves. The petiole has been used as a valuable characteristic in the separation of subspecies of H. helianthoides, both in terms of its length and shape (Fisher, 1957). In the case of the involucre, its bracts are of little value for separating taxa, as they are highly variable within a given species regarding shape, size, and pubescence. When the involucre has 2 or 3 series, its bracts are often foliaceous, both in plants from the United States and those from Mexico.



Picture 1: Leaves of some species from the Heliopsis genus. The leaves belonging to the species H. filifolia, H. helianthoides, and H. gracilis are images obtained from specimens deposited in the herbarium of the University of Texas in Austin; the leaves belonging to the species H. parviceps and H. parvifolia are images obtained from specimens deposited in the herbarium of the Instituto de Ecología A.C. in Pátzcuaro, Michoacán. The rest of the leaves are images of samples collected by the authors. 1. H. filifolia. 2. H. buphthalmoides. 3. H. procumbens. 4. H. annua. 5. H. parviceps. 6. H. helianthoides. 7. H. parvifolia. 8. H. novogaliciana. 9. H. anomala. 10. H. longipes. 11. H. sinaloensis. 12. H. gracilis.

The receptacle is generally convex to conical. The paleae are lanceolate, acute, and persistent after maturity, with red, purple, or yellow-brown tips, characteristics that together offer good criteria for separating certain taxa. The shape, texture, and size of the achenes is another valuable aid in species differentiation, as shown in Picture 2.



Picture 2: Achenes of some species from the Heliopsis genus. The images shown are photographs of materials collected by the authors and cited in the text. 1. H. annua. 2. H. anomala. 3. H. aff. novogaliciana locality Chavarría. 4. H. longipes locality Puerto de Tablas. 5. H. longipes locality San Cristóbal. 6. H. novogaliciana. 7. H. buphthalmoides. 8. H. procumbens. 9. H. sinaloensis.

Uses of Species from the Heliopsis Genus

The utility of these plants from an anthropocentric perspective has been limited to two species: Heliopsis helianthoides, marketed in the United States as an ornamental flower in gardening under the name "false sunflower," and Heliopsis longipes, with more diverse applications in Mexico, commonly known as chilcuague. The latter has a long tradition in indigenous herbal medicine, as indicated by its Nahuatl origin names. Some recorded names include: ichcha, cited in the translation of Francisco Hernández's work "De historia plantarum Novae Hispaniae"; chilcuán, meaning "snake chili"; chilmécatl, from chili, meaning "chili," and mécatl, meaning "rope," referring to the filiform roots and their spicy taste (Martínez, 1994); and chilicuau, a name used in the municipality of San Joaquín, Querétaro. Additionally, Martínez (1994) records pelitre or peritre as epithets likely assigned to it during the colonial era. Today, this species can be found in

medicinal herb stalls throughout much of the country under more modern names such as "golden root" and "Aztec root." Although the plant has not been found in pre-Columbian records, it can be found in early colonial literature, such as in extracts from Francisco Hernández's work "Quatro libros de la natvraleza" edited by Ximénez (1615). More recently, Noriega (1902), in his "Curso de historia de drogas," mentions it in relation to the replacement of African peritre: "... It is often replaced with the root of chilcuam or local peritre. Erygeron affinis (sic), which abounds around Mexico City and is likely the same abundant root in the Sierra de Querétaro, known as chilcuau" (sic). This uncertainty is probably related to the error made in identifying the roots when Acree and collaborators first isolated affinine, as mentioned later. Due to its insecticidal properties, it was one of the plants discovered by the United States during World War II, noting that extracts from these roots had the same degree of paralytic action and toxicity against flies and other insects as pyrethrum (Little, 1948). Aware of its use as an insecticide and its limited availability, the U.S. Department of Agriculture became involved in the search for other species of the same genus within its territory. From Heliopsis scabra Dunal, scabrine, an insecticidal alkamide present in its roots, was isolated (Jacobson, 1951). However, in preliminary tests, it was observed that scabrine, characterized by multiple unsaturated bonds and being more effective than pyrethrins in tests on houseflies, showed high toxicity to mammals (Roark, 1951). It is unknown which species was actually used in these studies, as that binomial is now obsolete. The specimens originally labeled as H. scabra, found in the herbarium of the University of Texas at Austin, have been reclassified, some as H. parvifolia and others as H. helianthoides (personal observation), and currently only the subspecies H. helianthoides ssp. scabra is recognized (Fisher, 1957). Consequently, the structure of scabrine N-isobutyl-1,3,7,9,14-octadodecapentadienamide has not been confirmed, nor has the isomerism of its bonds been resolved, as it has not been re-isolated. Fortunately, chilcuague was not driven to extinction during the export frenzy, although the wild population was severely reduced. According to Little (1948), the original collection site for chilcuague was the southern and southwestern area of the Sierra de Álvarez in San Luis Potosí. The same author located it in the northwestern part of Guanajuato in the Sierra Gorda, near the road from San Luis de la Paz eastward to Xichú, to Santa Catarina, and in the northern region of Querétaro. More than 50 years after this report, the known distribution is more or less the same. In these regions of Guanajuato and Querétaro, the cultivation of chilcuague has begun on a limited scale, along the edges of fields or in the shade of trees, in areas protected from grazing. However, today it is possible to observe plots of land dedicated entirely to the cultivation of this species. People who cultivate it indicate that propagation by cuttings, and in some cases by seeds, results in fully developed roots in two to three years. Currently, there is a government effort to establish chilcuague crops to ensure income for people in these regions. The price of this root can be satisfactory for producers, but there is not a sufficiently large and stable market outside the production region.

Taxonomic Knowledge of the Genus Heliopsis

Fisher (1957) provided a detailed account of the evolution of knowledge on the genus Heliopsis and, as a result of his review, recognized the following taxa: H. annua Hemsl., H. brachactis Standl. ex T. R. Fisher, H. buphthalmoides (Jacq.) Dunal, H. decumbens

Blake, H. filifolia S. Wats., H. gracilis (A. Gray) Nutt., H. helianthoides (L.) Sweet ssp. helianthoides, H. helianthoides (L.) Sweet ssp. occidentalis T. R. Fisher, H. lanceolata Blake, H. longipes (A. Gray) Blake, H. parviceps Blake, H. parvifolia A. Gray, H. procumbens Hemsl., and H. rubra T. R. Fisher. Later, in 1961, Fisher added one more species by describing H. pulchra, which was later recognized as part of H. buphthalmoides (McVaugh, 1984).

In 1958, Wilson described H. purpurea, based on collections by Hinton in Temascatepec, State of Mexico, and in Cutzamala, Guerrero. According to McVaugh (1984), H. purpurea is synonymous with H. parviceps, as the few collections observed with purple discs showed no significant differences beyond the yellow or purple coloration of the rays. Turner (1987) described H. novogaliciana, distinguishing it from H. procumbens mainly by the characteristics of the leaves, the appendages of the yellow anthers, and its geographical distribution. In the same work, he discussed the differences between H. brachactis and H. sinaloensis, the latter also being recognized as a new species.

The following year, Turner (1988) proposed replacing the name Heliopsis rubra T. R. Fisher with H. anomala (Jones) B. L. Turner. Finally, Díaz-Piedrahíta (1990) established the binomial H. oppositifolia (Lamarck) S. Díaz-Piedrahíta to replace H. buphthalmoides. However, it is worth noting that Heliopsis oppositifolia (L.) Druce, a name published in 1914 based on Rudbeckia oppositifolia (L.), takes precedence over Heliopsis oppositifolia (Lamarck) S. Díaz-Piedrahíta. The latter, being a later homonym, is illegitimate according to the International Code of Botanical Nomenclature (ICBN) and should not be used. In this work, considering this, we retain the epithet H. buphthalmoides (Jacq.) Dun.

Alkamides in the Genus Heliopsis

The chemical condensation of an acid with an amine results in the formation of an amide. The amide functional group is ubiquitous, found in all living organisms, constituting peptide bonds, which are the links between amino acids that form the primary structure of proteins, the molecules that are the foundation of life as we know it. Amides as secondary metabolites, however, are not as common. An interesting example of this group of compounds is the alkylamides or alkamides, which consist of approximately 70 known structures distributed across various levels of the Plant Kingdom.

Alkamides represent a unique class of natural products that, from a biogenic perspective, are formed by combining two different metabolic pathways. The acidic part originates from a fatty acid with a medium-to-long carbon chain, typically ranging from eight to eighteen carbons, usually aliphatic or linear. When this condenses with an amino acid and undergoes concomitant decarboxylation, an alkamide is produced. Alkamides whose acid chain is aliphatic can be separated into two groups, depending on the type of unsaturated bonds they present: olefinic alkamides, with at least one double bond, and acetylenic alkamides, with at least one triple bond (Greger, 1984). In the genus Heliopsis, only aliphatic and acetylenic alkamides have been found. Another

group includes amides with homo- or heterocyclic rings, which are particularly diverse in the Piperaceae family and include several alkaloids (Parmar et al., 1997).

Alkamides are considered bioactive compounds, meaning that a small amount of the compound elicits a notable response in receptor cells. H. longipes was the first species in which the presence of an olefinic alkamide was determined (Acree et al., 1945). However, the plant sample of roots subjected to laboratory analysis was mistakenly identified as Erigeron affinis, and thus the isolated amide was named affinine. The discovery of affinine, an insecticidal compound, in this species sparked interest due to its notable activity. Affinine is the major alkamide in the roots of this plant and is primarily responsible for the specific biological effects observed, including its local anesthetic action, organoleptic stimulation, as well as its insecticidal and bactericidal activity.

For a long time, affinine was considered the only bioactive component in this tissue, whose crude extract has been used to study its applications, which have recently expanded (Romero et al., 1989; Gutiérrez-Lugo et al., 1996), showing significant agronomic potential in the biological control of plant pathogenic bacteria and fungi (Ramírez-Chávez et al., 2000). The most detailed phytochemical analysis of H. longipes roots has revealed the presence of other bioactive compounds and the antimicrobial properties of some individual alkamides (Molina-Torres et al., 1995, 1996, 1999).

Alkamides, due to their bactericidal and fungicidal activity, likely constitute a defense element in plant species belonging to very distant taxonomic groups. These constitutive metabolites, or anticipins, have been found so far in ten families: Aristolochiaceae, Asteraceae, Brassicaceae, Convolvulaceae, Euphorbiaceae, Menispermaceae, Piperaceae, Poaceae, Rutaceae, and Solanaceae. In the subtribe Zinniinae of the Asteraceae family, alkamides are present in the genera Heliopsis, Acmella, Sanvitalia, and Salmea (Greger, 1984; Christensen and Lam, 1991; Christensen, 1992), and their study may reveal chemotaxonomic significance. In general, the species in which alkamides have been found have frequently been used by humans as medicinal plants.

Knowing that H. longipes contains other alkamides besides affinine as the major compound, an exploration was carried out to analyze their existence in other species of the genus Heliopsis and to assess whether the presence of these metabolites has taxonomic significance. From 1998 to 2001, collections of the genus Heliopsis were made in various regions of Mexico, including some locations in the Sierra Madre Occidental that were not previously mentioned for this genus.

Methods

Specimens of the Heliopsis genus corresponding to seven species were collected from ten localities. The collection sites, dates, and herbarium records of the studied species are presented in Table 2. The collected specimens were deposited in the Herbarium of the Instituto de Ecología A.C. in Pátzcuaro, Michoacán, with the records cited in Table 2. For the qualitative and quantitative analysis of the alkamides, only the roots were used. A 1-gram sample of fresh tissue from each specimen was taken, frozen in liquid

nitrogen, thoroughly homogenized in a mortar, and 5 ml of absolute ethanol was added. Each sample was subjected to sonication for 30 minutes at room temperature, then centrifuged at 3,000 rpm for 5 minutes. After separating the supernatant, the solvent was evaporated under a nitrogen flow. The residue was resuspended in 500 µl of absolute ethanol. This sample was analyzed using gas chromatography coupled with mass spectrometry (GC-MS) under previously described conditions (Ramírez-Chávez et al., 2000). The characterization of the alkamides was performed by comparing the retention time in the column and the mass spectrum of each eluted component. The concentration of each compound was estimated based on a calibration curve for affinine (N-isobutyl-2E,6Z,8E-decatrienamide).

Table 2: Specimens of the Heliopsis Genus Collected in This Study.

Species	Locality	Date	Number
H. annua	Cuitzeo-Moroleón Highway, junction to Chupícuaro, municipality of Cuitzeo, Michoacán. Lat. 19°59'49" N, Long. 101°09'18" W, alt. 1678 m.	27/07/99	J. Molina y A. García 104 (IEB)
H. anomala	Nacapule Canyon, at the base of the Sierra del Aguaje, municipality of Guaymas, Sonora. Lat. 28°01'28" N, Long. 111°06'09" W, alt. 150 m.	24/08/00	J. Molina y A. García 111 (IEB)
H. buphthalmoides	Cerro Machín, Oaxaca-Tuxtepec Highway, Oaxaca. Lat. 17°32'57.7" N, Long. 96°31'06" W, alt. 2841 m.	30/08/99	J. Molina 106 (IEB)
H. longipes	Puerto de Tablas, municipality of Xichú, Guanajuato. Lat. 21°14'20" N, Long. 100°05'19" W, alt. 2589 m.	05/05/99	J. Molina y A. García 101 (IEB)
	San Cristóbal, municipality of San Joaquín, Querétaro. Lat. 20°55'46" N, Long. 99°36'48" W, alt. 2478 m.	26/05/99	J. Molina 105 (IEB)
H. novogaliciana	Hacienda Coyotes, municipality of Pueblo Nuevo, Durango. Lat. 23°49'08.4" N, Long. 105°20'15.4" W, alt. 2368 m.	10/09/99	J. Molina, A. García y S. González 109 (IEB)
H. aff. Novogaliciana	Dirt road turnoff to Chavarría, 3.6 km from the junction with Durango–Mazatlán Highway, municipality of Pueblo Nuevo, Durango. Lat. 23°42'32.1" N, Long. 105°33'48.4" W, alt. 2484 m.	10/09/99	J. Molina, A. García y S. González 108 (IEB)
	2 km NW of El Tablón, municipality of Morelos, Chihuahua. Lat. 26°30'5" N, Long. 107°51'5" W, alt. 2200 m.	26/10/98	A. García 102 (IEB)
H. procumbens	Los Azufres, municipality of Hidalgo, Michoacán. Lat. 19°49'23" N, Long. 100°39'13" W, alt. 2760 m.	27/07/99	J. Molina y A. García 103 (IEB)
H. sinaloensis	Municipality of Imala, Culiacán, Sinaloa. Lat. 24°51'49.3" N, Long. 107°12'49.7" W, alt. 167 m.	22/08/00	J. Molina y A. García 110 (IEB)

Results

Of the seven collected species, only six were evaluated for the presence of alkamides in the roots: H. longipes, H. procumbens, H. novogaliciana, H. annua, H. sinaloensis, and H. buphthalmoides, with negative results for the last two species. In the case of H. anomala, a chemical evaluation was not conducted because only two specimens were collected—one of which was attempted to be kept alive for tissue culture establishment (without success), and the other was herbarium-preserved.

A total of nine distinct alkamides were found, along with a related structure, 2E,6Z,8E-boranyl triene decanoate (bornyl ester), as shown in Table 3. The dominant alkamide in each collection is highlighted in bold in each column.

From the samples analyzed, H. longipes was the species with the highest number of different compounds and the highest total alkamide content. The specimens collected from the two localities, Puerto de Tablas, Guanajuato, and San Cristóbal, Querétaro, showed some differences. The highest content was observed in the samples from Puerto de Tablas, with a total concentration of 9,369 µg/g of dry root weight distributed

across eight compounds. Meanwhile, 4,592 µg/g of dry root weight in nine compounds were obtained from the roots of plants from San Cristóbal. In this latter locality, the presence of N-isobutyl-2E,4Z,8Z,10E-tetraen-decamide, absent in the former, was observed. In both cases, N-isobutyl-2E,6Z,8E-trien-decamide (affinine) was the dominant alkamide, with 7,827 and 3,968 µg/g of dry root weight, respectively, followed by N-(2-methylbutyl)-2E,6Z,8E-trien-decamide, with 430 and 262 µg/g of dry root weight. The rest of the alkamides were present in smaller amounts and remained relatively proportional to affinine in both localities.

In terms of chain length, decamides were dominant, followed by undecamides. In both localities, the presence of the bornyl ester was detected, which, although not an amide, has an acid chain identical to affinine, and therefore its metabolism is linked. The presence of this compound is only known in the Heliopsis genus.

Table 3: Alkamides and Bornyl Decanoate Present in the Roots of Some Species of the Heliopsis Genus (µg/g dry weight).

Metabolito	*H. longipes*		*H. novogaliciana*	*H. aff. novogaliciana*		*H. procumbens*	*H. annua*	*H. buphthalmoides*	*H. sinaloensis*
	Pto. Tablas	San Cristóbal	Coyotes	Chavarría	El Tablón	Los Azufres	Cuitzeo	El Machín	Imala
N-isobutil-decamida	t	-	-	-	-	-	-	-	-
N-isobutil-2*E*,-monoen-decamida	t	-	-	-	-	-	-	-	-
N-isobutil-6*Z*,8*E*-dien-decamida	t	1	-	1	8	-	-	-	-
N-isobutil-2*E*,6*Z*,8*E*-trien-decamida	**7,827**	**3,968**	1	111	382	1	**8**	-	**t**
N-isobutil-2*Z*-en-8,10-diin-undecamida	173	29	-	-	-	-	-	-	-
N-(2-metilbutil)-2*E*,6*Z*,8*E*-trien-decamida	430	262	-	46	66	-	-	-	
N-isobutil-2*Z*,4*E*-dien-8,10-diin-undecamida	340	149	-			-	-	-	-
N-isobutil-2*E*-en-8,10-diin-undecamida	393	88	-	14	34	-	-	-	-
N-isobutil-2*E*,4*E*-dien-8,10-diin-undecamida	136	62	-	6	9	-	-	-	-
N-isobutil-2*E*,4*Z*,8*Z*,10*E*-tetraen-dodecamida	-	12	-	**177**	**594**	**23**	-	-	-
2*E*,6*Z*,8*E*-trien-decanoato de bornilo	70	21	**9**	-	-	-	-	-	-
N-(2-metilbutil)-2*E*,4*Z*,8*Z*,10*E*-tetraen-dodecamida	-	-	-	9	16	-	-	-	-
Total	9,369	4,592	10	364	1,109	24	8	0	t

Indicates the presence of the compound at levels below reliable quantitative evaluation under the equipment calibration conditions.

The alkamides of H. novogaliciana differ significantly between the studied localities. The plants from Hacienda Coyotes, Durango, show very low amounts of amides, with only trace levels of affinine and the presence of bornyl ester (9 µg/g of dry root weight), which was previously only observed in H. longipes. On the other hand, the H. aff. novogaliciana plants from the localities of Chavarría in Durango and El Tablón in Chihuahua show the same profile of seven compounds in total, with N-isobutyl-2E,4Z,8Z,10E-tetraen-dodecamide as the dominant compound (177 and 594 µg/g of dry root weight, respectively), followed by affinine (111 and 382 µg/g of dry root weight, respectively). In the specimens from Chavarría and El Tablón, dodecamides are dominant, and the bornyl ester is not observed.

The roots of H. procumbens present only two alkamides, both in low concentrations, with N-isobutyl-2E,4Z,8Z,10E-tetraen-dodecamide as the dominant one (23 µg/g of dry root weight), followed by trace levels of affinine. In H. annua, only affinine is observed at a low concentration (8 µg/g of dry root weight), while in H. buphthalmoides and H. sinaloensis, this group of metabolites could not be detected within the sensitivity range of the equipment used.

Discussion

As expected, the roots of H. longipes, known as chilcuague, have the highest affinine content, which justifies its traditional use, particularly in both localities of the Sierra Gorda. It is interesting to note that in the municipality of San Joaquín, Querétaro, and its surroundings, the name chilcuague is not recognized; instead, these roots are called chilicuau and are sold similarly to their counterparts in the municipalities of Xichú, San Luis de la Paz, Victoria, and other neighboring areas in the state of Guanajuato. This difference in nomenclature suggests that the people using it have had limited cultural exchange in this regard for a reasonably long time.

Furthermore, differences are observed between the plants from these two localities in the Sierra Gorda, which are geographically separated by approximately 60 km in a straight line. Both sites are isolated by lower altitude zones that are less humid and have conditions that appear to be unfavorable for the proper development of H. longipes. The total alkamide content is nearly double for the specimens from Guanajuato, which could be due to the more marked domestication of the species in this region or the age difference of the studied plants. The dodecamide with four unsaturations, N-isobutyl-2E,4Z,8Z,10E-tetraen-dodecamide, detected in the San Cristóbal locality, had not been observed in previous studies of H. longipes, as no alkamides with such a chain length were known in this species (Molina-Torres et al., 1995).

The data obtained suggest that there are already some genetic differences between the two populations mentioned, which could eventually lead to the formation of varieties within this species. In other genera of the Heliantheae tribe, such as Acmella, Echinacea, or Salmea, which contain alkamides, the dodecamides are the dominant compounds. Heliopsis longipes is the only known species that presents olefinic alkamides with only ten carbons, except for the aforementioned case in San Cristóbal. Additionally, the bornyl ester is included in this study as it is composed of the same

aliphatic chain of the 2E,6Z,8E-trienoic acid found in the major alkamides of Heliopsis species. This compound is present in the specimens from both H. longipes localities in the Sierra Gorda, was initially described in this species (Molina-Torres et al., 1995), and is now recorded for H. novogaliciana. To date, it has not been found outside this genus.

Regarding the plants identified as H. novogaliciana and H. aff. novogaliciana, significant differences exist in the alkamide profiles of their roots between the former and the latter. The specimens collected at Hacienda Coyotes in Durango contain only small amounts of two of the compounds studied: affinine and bornyl ester. The H. aff. novogaliciana specimens from the localities of Chavarría in Durango and El Tablón in Chihuahua contain the same seven alkamides, with the tetrainsaturated dodecamides being dominant. Although the material from Chihuahua contains three times the total amount of alkamides as that from Durango (1,109 vs. 364 µg/g of dry root weight), the possibility that the analyzed samples came from plants of different ages cannot be ruled out. This collection point, El Tablón in Chihuahua, is a relatively isolated place far from previously recorded sites for this genus.

Finally, the roots of the other four species analyzed (H. procumbens, H. annua, H. sinaloensis, and H. buphthalmoides) only reveal alkamide levels with total values below 25 µg/g of dry root weight. H. procumbens contains only 24 µg/g of the dodecamide that predominates in H. aff. novogaliciana, while H. annua contains only 8 µg/g of dry weight, and the other two species show undetectable levels of these metabolites.

Considering the amount of alkamides in the roots of the studied plants, they can tentatively be subdivided into two groups: species with high levels and diversity of alkamides, which include H. longipes and H. aff. novogaliciana from the localities of Chavarría and El Tablón, and a second group with low amounts and variety of these compounds, which includes H. novogaliciana, H. procumbens, H. annua, H. sinaloensis, and H. buphthalmoides. The data presented so far show significant differences between the various taxa in the Heliopsis genus, raising some questions and indicating the need to expand the study, as well as explore the use of new research strategies, such as different DNA-based techniques. A current study is being developed with molecular markers based on RAPDs and ITSs to strengthen the proper taxonomic placement of these species.

Acknowledgments

The authors wish to thank Dr. Jerzy Rzedowski for his help in the identification of species; Dr. Socorro González Elizondo for her support in fieldwork and for reviewing a preliminary manuscript; and to CONACYT agreement: 26398N, Sistema de Investigación Miguel Hidalgo (SIHGO) agreement 97810, and Fundación Guanajuato Produce agreement 31/98 for the financial support provided for this contribution.

Cited Literature

Acree, F., M. Jacobson, and H. L. Haller. 1945. The structure of affinin, the insecticidal amide from Erigeron affinis D.C. Journal of Organic Chemistry 10: 449-451.

Blake, S. 1924. New American Asteraceae. Heliopsis longipes (A. Gray) Blake. Contributions from the U.S. National Herbarium 22: 608-609.

Bremer, K. 1994. Asteraceae: cladistics & classification. Timber Press. Portland. 752 pp.

Christensen, L. 1992. Acetylenes and related compounds in Anthemideae. Phytochemistry 31: 7-49.

Christensen, L. and L. Lam. 1991. Acetylenes and related compounds in Heliantheae. Phytochemistry 30: 11-49.

Díaz-Piedrahíta, S. 1990. On the validity of two names of Colombian asteraceae and their correct typification. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 67: 645-648.

Fisher, T. R. 1954. A new species of Heliopsis from Baja California, Mexico. Madroño 12: 152-155.

Fisher, T. R. 1957. Taxonomy of the genus Heliopsis (Compositae). Ohio Journal of Science 57: 171-191.

Fisher, T. R. 1961. A new species of Heliopsis from Mexico. Ohio Journal of Science 61: 178-179.

Gray, A. 1879. Characteristics of some new species of Compositae. Proceedings of the National Academy of Sciences, USA 15: 25-51.

Greger, H. 1984. Alkamides: structural relationships, distribution, and biological activity. Planta Medica 50: 366-375.

Gutiérrez-Lugo, M. T., T. Barrientos-Benítez, B. Luna, R. M. Ramírez-Gama, R. Bye, E. Linares, and R. Mata 1996. Antimicrobial and cytotoxic activities of some crude drug extracts from Mexican medicinal plants. Phytomedicine 2(4): 341-347.

Jacobson, M. 1951. Constituents of Heliopsis species. I. Scabrin, an insecticidal amide from the roots of H. scabra Dunal. Journal of the American Chemical Society 73: 100-103.

Little, E. 1948. El chilcuague (Heliopsis longipes). Planta insecticida. Boletín de la Sociedad Botánica de México 7: 23-27.

Martínez, M. 1933. Las plantas medicinales de México. 6th ed. Editorial Botas. Mexico City. 656 pp.

McVaugh, R. 1984. Heliopsis Pers. In: Flora Novo-Galiciana. Compositae. 12: 496-502.

Molina-Torres, J., R. Salgado-Garciglia, E. Ramírez-Chávez, and R. del-Rio. 1995. Presence of the bornyl ester of deca-2E,6Z,8E-trienoic acid in Heliopsis longipes roots. Journal of Natural Products 58: 1590-1591.

Molina-Torres, J., R. Salgado-Garciglia, E. Ramírez-Chávez, and R. del-Rio. 1996. Purely olefinic alkamides in Heliopsis longipes and Acmella (Spilanthes) oppositifolia. Biochemical Systematics and Ecology 24: 43-47.

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

Noriega, J. 1902. Curso de historia de drogas. Instituto Médico Nacional. Oficina Tipográfica de la Secretaría de Fomento, Mexico City. 837 pp.

Parmar, V. S., S. C. Jain, K. S. Bisht, R. Jain, P. Taneja, A. Jha, O. D. Tyagi, A. K. Prasad, J. Wengel, C.E. Olsen, and P.M. Bool 1997. Phytochemistry of the genus Piper. Phytochemistry 46: 597-673.
Ramírez-Chávez, E., L. Lucas-Valdez, G. Virgen-Calleros, and J. Molina-Torres. 2000. Fungicidal activity of affinin and crude extract of Heliopsis longipes roots against two species of Sclerotium. Agrociencia 34: 207-217.
Roark, R.C. 1951. Scabrin. Soaps and sanitary chemicals 27: 125,137.
Romero, C. M., A. R. Del-Castillo, A. C. Martínez, and C. J. Calderón. 1989. Preliminary studies on the antibacterial, insecticidal, and toxicological effects of the chilcuan root Heliopsis longipes. Veterinaria 20: 151-156.
Rzedowski, G. C. de, J. Rzedowski, and collaborators. 2001. Flora fanerogámica del Valle de México. 2nd ed., Instituto de Ecología, A.C. and Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Pátzcuaro. 1406 pp.
Turner, B. L. 1987. Two new species of Heliopsis (Asteraceae) from northwestern Mexico. Phytologia 63: 1-3.
Turner, B. L. 1988. Comments upon and new combinations in Heliopsis (Asteraceae, Heliantheae). Phytologia 64: 337-339.
Wilson, P. G. 1958. Contributions to the flora of tropical America: LXIII. Kew Bulletin 164: 155-165.
Ximénez, F. 1615. Qvatro libros de la natvraleza. Facsimile edition printed in Morelia, 1888. 300 pp.