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Research article

Caracterización ecológica y fitoquímica de poblaciones naturales de *Galphimia* spp. en el estado de Aguascalientes

Ecological and phytochemical characterization of *Galphimia* spp. natural populations in the state of *Aguascalientes*

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Abstract

The *Galphimia* genus of the Malpighiaceae family contains 24 species, among the most representative are *Galphimia gracilis*, *Galphimia angustifolia* and *Galphimia glauca*. In México the latter distributes in the Central and Northeastern regions, where it exhibits anti-inflammatory, sedative, and anxiolytic properties. However, the presence and accumulation of bioactive metabolites such as galfimins, a type of triterpene, present in samples collected in the states of *Guanajuato* and *Querétaro*, and galphimins and galphimidins from the states of *Guerrero* and *Morelos*, have been reported in a few wild communities in Mexico. The objective of the present study was to generate ecological, morphological and phytochemical information associated with natural populations of *Galphimia* spp. in four sites at the state of *Aguascalientes* (*Aguascalientes*, *Jesús María*, *Calvillo* and *San José de Gracia*). Ecological conditions were assessed through an inventory for site recognition; morphological conditions with the methodology proposed by the International Plant Genetic Resources Institute and specialized taxonomic keys; and phytochemistry with plant samples from each population to determine the presence of triterpenes using automated thin layer and high-resolution liquid chromatographic techniques. The sites with the presence of *Galphimia* spp. in the state showed some degree of disturbance and environmental differences in soil and vegetation. Morphological and phenological characteristics did not detect differences between populations. The phytochemical profile did not show the presence of known galfimins and galphimidins in the populations. This is the first report on phytoecological and phytochemical analyses of natural populations of *Galphimia* spp. in *Aguascalientes*. Larger studies are required to identify *Galphimia* species in the state of *Aguascalientes* and analyze the biological activity of some bioactive compounds that were detected, which could be of pharmacological and industrial interest.

Key words: *Aguascalientes*, galphimidins, galphimins, *Galphimia* Cav., wild populations, triterpenes.

Resumen

El género *Galphimia*, familia Malpighiaceae, incluye 24 especies, y entre las más representativas están: *Galphimia gracilis*, *Galphimia angustifolia* y *Galphimia glauca.* Esta última se distribuye en las zonas central y noreste de México; se le atribuyen propiedades antiinflamatorias, sedantes y ansiolíticas. Asimismo, se ha identificado la presencia y acumulación de metabolitos bioactivos como los triterpenos, denominados galfiminas, en muestras recolectadas en Guanajuato y Querétaro; galfiminas y galfimidinas en ejemplares de Guerrero y Morelos. Sin embargo, hay pocos registros en comunidades silvestres. El objetivo del presente estudio fue generar información ecológica, morfológica y fitoquímica asociada a cuatro poblaciones naturales de *Galphimia* spp. del estado de Aguascalientes (Aguascalientes, Jesús María, Calvillo y San José de Gracia). En cada población se evaluaron condiciones ecológicas mediante inventario para reconocimiento del sitio; morfológicas con la metodología propuesta por el *International Plant Genetic Resources Institute* y claves taxonómicas; y fitoquímicas con muestras vegetales mediante técnicas cromatográficas en capa fina automatizada y de líquidos de alta resolución. Los cuatro sitios con presencia de *Galphimia* spp. mostraron algún grado de disturbio y diferencias ambientales en suelo y vegetación. En las características morfológicas y fenológicas no hubo diferencias entre poblaciones. El perfil fitoquímico no reveló presencia de galfiminas y galfimidinas citadas en otras poblaciones. Este es el primer análisis fitoecológico y fitoquímico de poblaciones naturales de *Galphimia* spp. en Aguascalientes. Se requieren estudios más amplios para identificar las especies de *Galphimia* en el estado y analizar la actividad biológica de algunos compuestos bioactivos detectados que podrían ser de interés farmacológico e industrial.

Palabras clave: Aguascalientes, galfimidinas, galfiminas, *Galphimia* Cav., poblaciones silvestres, triterpenos.

Introduction

The *Galphimia* Cav. genus, belongs to the Polygales order, Malpighiaceae family (Stevens, 2024); 24 species of this genus are present in Mexico (Villaseñor, 2016), some of them of scientific and commercial interest due to their phytochemical derivatives, such as *Galphimia glauca* Cav. (Sharma et al., 2012a) distributed in central states of the country (Gesto-Borroto et al., 2019), mainly those located in the biogeographic provinces of the Southern Highlands, Trans-Mexican Volcanic Axis, Balsas Depression, and Southern *Sierra Madre* (Espinosa et al., 2008).

In terms of its phytochemical profile, the first nor-dry-triterpene discovered, isolated from *G. glauca* was galphimin B, in specimens collected in *Dr. Mora*, *Guanajuato*, with sedative activity (Tortoriello & Lozoya, 1992). Subsequently, other types of triterpenes were registered: A-C and galphimidin with antiprotozoal properties (del Rayo et al., 2002), galphimins A-F with sedative and anxiolytic activity (Cardoso et al., 2004), and anti-inflammatory activity was observed in methanolic extracts of *Galphimia* populations from the states of *Guanajuato*, *Querétaro*, *Jalisco*, and *Morelos* (Cardoso-Taketa et al., 2008). The anti-inflammatory activity of the components was described by González-Cortazar et al. (2014), and recently the anti-inflammatory action of two galphimidins was studied in depth (León-Álvarez et al., 2024).

G. glauca is cited in the without risk category (NOM-059-SEMARNAT-2010, 2010), associated with areas of anthropic disturbance, with poor soils and disturbed vegetation (Siqueiros et al., 2017). Gesto-Borroto et al. (2019) identified four *Galphimia* species from *Guanajuato*, *Querétaro*, *Hidalgo*, *Morelos* and *San Luis Potosí*; to determine the species of *Galphimia*, the authors used six DNA markers (matK, rbcL, rpoC1, psbA-trnH, ITS1, and ITS2) in combination with the chemical profiles of each population, using thin layer chromatography.

The wild populations of *Aguascalientes* are botanically catalogued as *Galphimia glauca* (Siqueiros et al., 2017; Villaseñor, 2016), but information on their distribution and biological activity is scarce and on their phytochemical compounds is null. However, and given the discussion and confusion about the identification of *Galphimia* taxa collected in Mexico, based on botanical descriptors or DNA markers, in this paper specimens collected for study as *Galphimia* spp. will be considered. In this regard, DNA barcoding analyses have revealed the misclassification of many species of *Galphimia* that have been identified as *G. glauca*. The objective of this research was to generate ecological, morphological, and phytochemical information associated with four natural populations of *Galphimia* spp. present in the state of *Aguascalientes* (*Aguascalientes*, *Jesús María*, *Calvillo*, and *San José de Gracia*).

Materials and Methods

Ecological characterization of *Galphimia* spp.

The study area was selected based on those spots within the state of *Aguascalientes* where the presence of *Galphimia* spp. was registered, including the municipalities of *Aguascalientes* (AGS), -21°53’01.0206” N and 102°25’22.6204” W; *Jesús María* (JM), -21°54’24.63” N and 102°31’59.19” W; *Calvillo* (CAL), -21°53’30.56” N and 102°35’39.37” W, and *San José de Gracia* (SJG), -22°9’22.46” N and 102°32’27.22” W (Siqueiros, 2024). Using model eTrex 3 Garmin® equipment, unstratified sampling polygons were drawn in variable areas (one per location) recommended for this type of study (McRoberts et al., 1992). The data were subsequently processed in Arcmap 10.8.1, using vector layers of biogeographic regions (Espinosa et al., 2008), and vegetation type (Siqueiros et al., 2017).

Personnel from the herbarium of the *Universidad Autónoma de Aguascalientes* (Autonomous University of *Aguascalientes*) (HUAA) herborized (Lot & Chiang, 1986), identified and authenticated the specimens collected by site, using specialized taxonomic keys as proposed by Rzedowski (2006), assigning them collection accession numbers. The species occurring in each site were documented using the procedure described by Romahn and Ramírez (2010).

Five samples of 100-200 g of soil were obtained from each site and subsequently blended to form a 1 kg composite sample according to the criteria of the Mexican Official NORM NOM-021-RECNAT-2000 (2001). Climatological data were drawn from the geographical and environmental maps’ digital system of *Instituto*

Nacional de Estadísticas y Geografía (Inegi, 2022). An inventory was made of specimens presumably of *G. glauca* located by site, and five individuals were selected by simple sampling.

The morphological characteristics (height, diameter, number of flowers per axil, as well as leaf, fruit and seed dimensions and their physical characteristics) were recorded in keeping with the protocol of Romahn and Ramírez (2010). The characterization variables proposed by the International Board for Plant Genetic Resources Institute (IBPGR, 1980) for tropical species were also used. During one year (July 2021-July 2022), the phenological stages of the selected plants were monitored *in situ*, and the behavior and reproduction cycle of wild specimens were documented. Data for each site were recorded every 22 days; the information thus gleaned was used to determine the number of days necessary for the beginning of the foliation, flowering, and fruiting phases, according to Fournier's methodology (1974). In addition, a database was integrated and complemented with photographic evidence of each site.

Phytochemical characterization of *Galphimia* spp.

Sample collection

In March 2023, five specimens per site were selected, classified as *G. glauca* of reproductive age at leaf stage and as being healthy, vigorous, and free of pests and diseases; the criteria of the manual for the establishment of forest germplasm production units were followed (Comisión Nacional Forestal [Conafor], 2016).

Freshly mature leaves from the middle part of the plant were collected from the specimens to form one sample per site; the material was placed in labeled paper bags for shipment to the *Laboratorio de Análisis de Suelo*, Agua *y Nutrientes Vegetales* of the *Universidad Autónoma de Aguascalientes* (Soil, Water and Plant Nutrient Analysis Laboratory of the Autonomous University of *Aguascalientes*) for preparation and subsequent shipment as ethanoic extracts for chromatographic analysis to the *Laboratorio de Plantas Medicinales de la Universidad Autónoma del Estado de Morelos* (Medicinal Plants Research Laboratory of the Autonomous University of the State of Morelos).

Extract preparation

Ethanolic extracts were prepared from the plant samples of the four populations, according to the methodology described by Cardoso-Taketa et al. (2008) with certain modifications. The samples were dried for seven days in a cool, dry place out of the direct sunlight and pulverized with a mortar. 1 mL of 96 % ethanol was added to each sample of the completely dry and pulverized material (500 mg), shaking the mixture for 30 seconds at 600 rpm in a model 2 Genie® vortex, sonicating it for 10 minutes in a model D150H Ultrasonic Cleaner MRC®, and then centrifuging it in a model 5702 Eppendorf® centrifuge at 3 000 rpm for 5 minutes. The supernatant was recovered, and the residue containing the sediment was reprocessed three times to achieve a final volume of approximately 2.5 mL of extract per sampled site.

Thin-Layer Chromatography (TLC) Analysis

A TLC was performed using silica plates (60 UV_{254} silica gel, particle size 5 μm, 0.2 mm, on aluminum support, Merck®) and elution was carried out with a mobile phase of chloroform and ethyl acetate (1:2 v/v). The TLC plates were visualized under UV light at 254 nm and 366 nm (Gesto-Borroto et al., 2019). They were then developed with a 0.1 % vanillin solution in H_2SO_4 and heated in a model SP131325 TSU® electric grill at 110 °C until the presence of metabolites, mainly triterpenes, was observed. In order to compare and analyze the extracts, a standard sample of galphimins triterpenes isolated from a *G. glauca* population of *Dr. Mora*, *Guanajuato* state (GM), was utilized, together with a sample from the population of *Tepoztlán*, *Morelos* state (TM), where galphimins do not occur. The standards galphimidin (1) and galphimidin B (2), isolated in previous studies by Cardoso et al. (2004) and León-Álvarez et al. (2024), were obtained from the latter population.

High-Performance Liquid Chromatography (HPLC)

The chromatographic profile of the ethanolic extracts of the collected *Galphimia* spp. populations was performed with the HPLC technique under the conditions described by Cardoso et al. (2004). The analysis was carried out with a model LC-Net ll/ADC JASCO® equipment (50/60 HZ), equipped with a C18 analytical column (Capcell Pak®; 5 μm, 4.6×250 mm).

The elution system was isocratic, using a mixture of acetonitrile and water (45:55 v/v) with a flow of 0.8 mL min^{-1}. 20 μL of each sample was injected at a concentration of 3 mg mL^{-1} and the detection of metabolites was done with a model 2 CAMAG TLC Visualizer® UV detector set at 254 nm.

The extracts obtained were analyzed in parallel with an extract of *Galphimia glauca* from *Dr. Mora*, *Guanajuato* (GM), previously characterized and recognized for its production of galphimins (Cardoso et al., 2004; Gesto-Borroto et al., 2019).

Results and Discussion

Ecological characterization of *Galphimia* spp.

The sites with *Galphimia* spp. exhibited some differences in the plant communities that form and interact with them, as well as in certain environmental conditions (Table 1). These data are consistent with information from the digital system of geographical and environmental maps of Inegi (2022). In *Aguascalientes*, *Galphimia* spp. were found to be distributed in two of the three biogeographical regions cited by Espinosa et al. (2008): *Aguascalientes*, *Calvillo* and *Jesús María* for the Southern *Altiplano*, and *San José de Gracia* for the *Sierra Madre Occidental*, where they are part of the vegetation of a floristic transition zone in the state, as mentioned by Rzedowski (2006). The characterization of the wild specimens of *Galphimia* spp. sampled in the four geographical areas showed similarities in morphology and phenology between them that rule out any mutation or speciation, in addition to expressing similarities between the attributes of the species and those of the authenticated specimens (eFloraMEX, 2023) (Table 2); however, no biotechnological or genetic verification was carried out, unlike in the study by Sharma et al. (2012b).

Table 1. Environmental descriptors by site.

Descriptor	*Aguascalientes*	*Jesús María*	*Calvillo*	*San José de Gracia*	*Guanajuato* (revised site)
1	2 062 m	2 173 m	2 148 m	2 514 m	2 120 m
2	*Opuntia* spp., *Mimosa monancistra* Benth., *Acacia schaffneri* (S. Watson) F. J. Herm., *Prosopis laevigata* (Humb. & Bonpl. *ex* Willd.) M. C. Johnst.	*Quercus potosina* Trel., *Opuntia* spp., *Mimosa monancistra* Benth., *Acacia schaffneri* (S. Watson) F. J. Herm., *Prosopis laevigata* (Humb. & Bonpl. *ex* Willd.) M. C. Johnst., *Yucca filifera* Chabaud	*Opuntia* spp., *Mimosa monancistra* Benth., *Acacia schaffneri* (S. Watson) F. J. Herm., *Prosopis laevigata* (Humb. & Bonpl. *ex* Willd.) M. C. Johnst., *Forestiera phillyreoides* (Benth.) Torr., *Yucca filifera* Chabaud	*Arctostaphylos pungens* Kunth, *Quercus potosina* Trel., *Juniperus deppeana* Steud.	*Quercus eduardii* Trel., *Quercus laeta* Liebm., *Opuntia* spp., *Ambrosia camphorata* (Greene) W. W. Payne, *Myrtillocactus geometrizans* (Mart. *ex* Pfeiff.) Console
3	Complex formation of herbaceous, shrubs and succulents	Complex formation of herbaceous, shrubs and succulents	Xerophytic scrub	*Chaparral*	Complex formation of herbaceous, shrubs and succulents
4	Xerophytic scrub	Oak forest	Temperate/crasicaule scrub	Oak-pine forest	Thorn scrub
5	Xerophyll	Xerophyll	Temperate	Temperate	Xerophyll
6	Shrub	Shrub	Shrub	Arboreal	Shrub
7	Leptosol	Leptosol	Young with little agricultural development, regosol	Rainfall-dependent agriculture, phaeozem	Yermosol-regosol
8	Urbanization	Overgrazing	Deforestation	Overgrazing	Erosion
9	*Morcinique* river	*Calvillo* river	*Calvillo* river	*San Pedro* river	Does not count
10	18-21 °C	18–21 °C	18–21 °C	14-18 °C	17.8 °C
11	400-500 mm	500-700 mm	500-700 mm	500-700 mm	664 mm
12	75-90 %	50-75 %	50-75 %	50-75 %	No data
13	Southeast	Southeast	Southeast	Southeast	No data
14	49-64 %	16-25 %	25-36 %	25-36 %	8-20 %
15	20 %	5 %	10 %	10 %	No data
16	20 %	25 %	20 %	20 %	No data
17	5 %	10 %	5 %	5 %	No data
18	5 %	5 %	5 %	5 %	No data

19	45 %	50 %	55 %	55 %	No data
20	5 %	5 %	5 %	5 %	1.4 %
21	Circulating water	Circulating water	Circulating water	Circulating water	No data

1 = Altitude of the location (m); 2 = Plant community; 3 = Plant formation; 4 = Vegetation type; 5 = Vegetation group; 6 = Physiognomy; 7 = Edaphology; 8 = Degradation; 9 = Hydrology; 10 = Mean temperature (°C); 11 = Average precipitation (mm); 12 = Stratum coating (%); 13 = Exposure; 14 = Slope (%); 15 = Hard rock and blocks (%); 16 = Rocks (%); 17 = Sand (%); 18 = Fine earth (%); 19 = Vegetation (%); 20 = Organic matter (%); 21 = Submersion.

Table 2. *Galphimia glauca* Cav. on-site descriptors.

Descriptor	*Aguascalientes*	*Jesús María*	*Calvillo*	*San José de Gracia*	*Guanajuato* (reviewed specimen)
1	Small shrub (<5m)	Small shrub (<5m)	Small shrub (<5m)	Small shrub (<5m)	Small shrub (<5m)
2	1.20 m	1.15 m	1.02 m	1.27 m	1.5 m
3	1.37 cm	1.17 cm	1.05 cm	1.35 cm	No data
4	Monopodic	Monopodic	Monopodic	Monopodic	Monopodic
5	Numerous primary branches	Numerous primary branches	Numerous primary branches	Numerous primary branches	Numerous primary branches
6	Semierect	Semierect	Semierect	Semierect	Erect or scandent
7	Greenish	Greenish	Greenish	Greenish	No data
8	Ovate	Ovate	Ovate	Ovate	Ovate
9	Sharp	Sharp	Sharp	Sharp	Sharp
10	5 cm in average when mature	5.3 cm in average when mature	5.1 cm in average when mature	5.2 cm in average when mature	5 cm
11	2.3 cm in average when mature	2.1 cm in average when mature	2 cm in average when mature	2 cm in average when mature	1.5 cm
12	9 mm in average per year	7 mm in average per year	5 mm in average per year	5.1 mm in average per year	9 mm
13	Dark brown	Dark brown	Dark brown	Dark brown	No data
14	Green	Green	Green	Green	No data
15	Axillary	Axillary	Axillary	Axillary	Axillary
16	23 in average	27 in average	25 in average	23 in average	No data
17	Included	Included	Included	Included	Included
18	10 in average per	10 in average per	10 in average per	10 in average per	No data

	flower	flower	flower	flower	
19	Brown	Brown	Brown	Brown	Brown
20	Rounded	Rounded	Rounded	Rounded	Rounded
21	Coriacea	Coriacea	Coriacea	Coriacea	No data
22	Yes	Yes	Yes	Yes	Yes
23	3.0 mm in average when mature	2.9 mm in average when mature	3.0 mm in average when mature	3.0 mm in average when mature	3.2 mm
24	3.0 mm in average when mature	3.0 mm in average when mature	3.0 mm in average when mature	3.0 mm in average when mature	2.27 mm
25	2 mm in average when mature	2 mm in average when mature	2 mm in average when mature	1.9 mm in average when mature	2.23 mm
26	2 mm in average when mature	2 mm in average when mature	2 mm in average when mature	1.6 mm in average when mature	1.8 mm
27	Brown	Brown	Brown	Brown	No data
28	Rounded	Rounded	Rounded	Rounded	Globose
29	April-June	April-June	April-June	April-July	Perennial
30	July-October	July-October	July-October	July-October	August-September
31	August-October	August-October	August-October	August-October	August-October
32	September-December	September-December	September-December	September-December	No data

1 = Plant habitat; 2 = Average height (m); 3 = Stem diameter (cm); 4 = Vegetative development; 5 = Branching habit; 6 = Insertion of branches; 7 = Young leaf color; 8 = Leaf shape; 9 = Leaf apex shape; 10 = Leaf length; 11 = Leaf width; 12 = Petiole length; 13 = Petiole color; 14 = Young shoot color; 15 = Inflorescence; 16 = Flowers per axil; 17 = Insertion of anthers; 18 = Number of stamens; 19 = Fruit color; 20 = Fruit shape; 21 = Endocarp texture; 22 = Calyx limb; 23 = Fruit length; 24 = Fruit width; 25 = Seed length; 26 = Seed width; 27 = Seed color; 28 = Seed shape; 29 = Foliation; 30 = Flowering; 31 = Fruiting; 32 = Maturation.

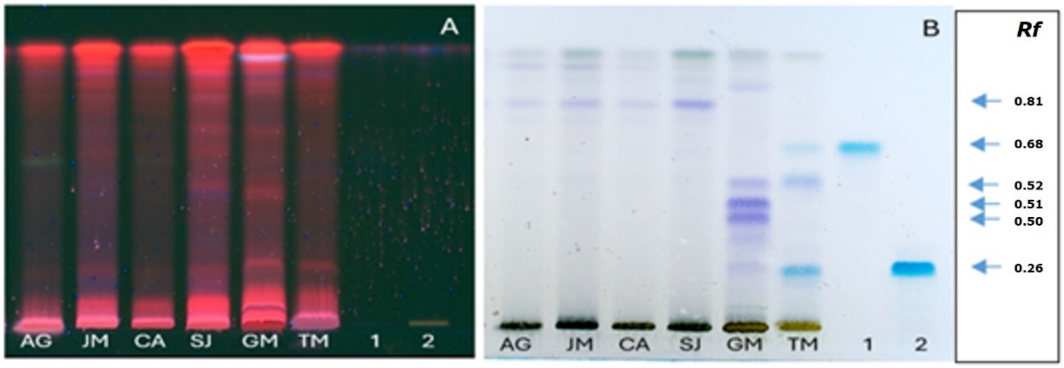
The four sites with *Galphimia* spp. were observed to show anthropic alterations, such as overgrazing, fires and land use change, which confirm the establishment of the species in sites with disturbed vegetation and degraded environments (Siqueiros et al., 2017). The general data concerning the characterization of the collection, the environmental descriptors and the specimens located by site are shown in Tables 1 and 2, respectively.

Phytochemical characterization of *Galphimia* spp. by TLC

The chemical profile of ethanolic extracts obtained from individuals collected in the four localities of *Aguascalientes* revealed the presence of violet bands in a Thin-Layer Chromatography (TLC) when treated with vanillin/H_2SO_4/110 °C. This coloration is characteristic of bioactive nor-triterpenes of the galphimins family, recorded in populations producing this compound (Gesto-Borroto et al., 2019; Sharma et al., 2012). In contrast, those from non-galphimins-producing populations show a blue coloration (León-Álvarez et al., 2024).

The presence of these unidentified compounds with lower polarity compared to the galphimins and galphimidins of the *Guanajuato* and *Morelos* extracts, respectively, suggests that the populations have unique triterpene profiles. In particular, a major compound was detected in the four sampling sites, with a Retention factor (*Rf*) of 0.8 and a violet coloration, which raises interesting perspectives on the structure and potential biological activity, given that it has not been previously documented in Mexican *Galphimia* spp.

The TLC triterpene profile of the samples from *Aguascalientes* is more simplified compared to that of individuals collected in *Guanajuato*, *Querétaro* and *Morelos*. It is noteworthy that the compound with *Rf* 0.8 is exclusive of the four populations of *Galphimia* from *Aguascalientes* and differs from those obtained in the population of *Guanajuato*, which exhibit an *Rf* of 0.5. In addition, the *Aguascalientes* extracts do not contain the galphimidins identified in the *Morelos* population, since galphimidin and galphimidin B exhibit an *Rf* of 0.6 and 0.2, respectively (Figure 1).



A = Observed under UV light at 366 nm; B = Developed with vanillin/H_2SO_4/110 °C; *Rf* = Retention factor; AG = *Aguascalientes*; JM = *Jesús María*; CA = *Calvillo*; SJ = *San José de Gracia*; GM = *Dr. Mora*, *Guanajuato* (positive control for galphimins and negative control for galphimidins); TM = *Tepoztlán*, *Morelos* (positive control for galphimidin [1] and galphimidin B [2] and negative for galphimins).

Figure 1. Automated thin layer chromatographic profiling (CAMAG) of extracts of wild specimens from populations of the states of *Aguascalientes*, *Guanajuato* and *Morelos* in normal phase silica and mobile phase $CHCl_3$:AcOEt (1:2).

In order to characterize the compound with *Rf* 0.8, it will be necessary to purify it by chromatographic methods and to analyze it with ^1H and ^{13}C nuclear magnetic resonance, as well as to determine its molecular weight by mass spectrometry.

The TLC analysis of the *Aguascalientes* extracts also failed to show bands corresponding to the blue-revealing compounds, such as galphimidin and galphimidin B. These compounds are present in the population of *Tepoztlán*, *Morelos* (TM) and correspond to nor-triterpenes, originally isolated from the populations of *Ayutla*, *Guerrero* (del Rayo et al., 2002) and *Cuernavaca*, *Morelos* (Rios et al., 2020), respectively. Both compounds have anti-inflammatory activity in RAW 264.7 macrophage cells, and inhibit nitric oxide production (León-Álvarez et al., 2024).

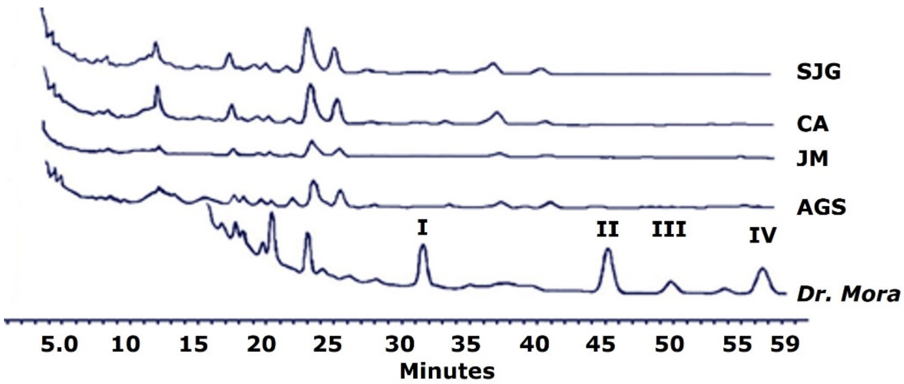
The TLC profiles —particularly the absence of galphimins cited in the *Guanajuato* population and of galphimidins identified in *Morelos*— clearly differentiate the populations of *Aguascalientes* from those of *Dr. Mora*, *Guanajuato* (GM) and *Tepoztlán*, *Morelos* (TM), as shown in Figure 1. Previous studies of various populations of *Galphimia* spp. suggest that differences in metabolite production may be related to genetic and environmental factors (Gesto-Borroto et al., 2019).

A metabolomic analysis performed by Sharma et al. (2012b) on *Galphimia* spp. populations collected in Mexico showed that the population from *Zapopan*, *Jalisco*, has a distinct chemical profile compared to other populations. Considering that the populations collected in *Jalisco* are closer to those of *Aguascalientes*, it would be interesting to explore the degree of chemical similarity between the populations of both regions.

On the other hand, phylogenetic analysis based on DNA barcoding methodology revealed that the populations of *Galphimia* spp. from *Morelos*, *Guerrero* and *Jalisco* are grouped in the same cluster, while those from *Guanajuato* and *Querétaro* form another cluster corresponding to *Galphimia glauca*. Given the geographic proximity between the populations of *Jalisco* and *Aguascalientes*, it would be relevant to investigate whether they share phylogenetic similarities, since neither of them produces galphimins (Sharma et al., 2012a).

High Performance Liquid Chromatography (HPLC) Analysis

The HPLC chromatographic profiles of the ethanolic extracts of the *Aguascalientes* populations showed several peaks with retention times (R_t) of 23.0 to 26.0 min. In contrast, the reference extract from the population of *Dr. Mora*, *Guanajuato*, exhibited R_t peaks of 32.5, 46.5, 50.0, 54.0 and 58.0 min, corresponding to the I-V peaks described by Cardoso et al. (2004) (Figure 2). Peak II, the most abundant was identified as the diastereomeric mixture of galphimins B and F.



SJG = *San José de Gracia*; CA = *Calvillo*; JM = *Jesús María*; AGS = *Aguascalientes*; *Dr. Mora* (*Guanajuato*).

Figure 2. HPLC of ethanolic extracts of four plant populations of *Galphimia* spp. collected in *Aguascalientes* and a galphimins-producing population (Peaks I-V) of *Dr. Mora*, *Guanajuato*.

It is important to note that the chromatograms of the four samples from the *Aguascalientes* sites showed majority and minority peaks. The retention times of the majority peaks were in the range of 23.1 and 25.5 min, while those of the minority peaks were between 36.9 and 41.0 min, so they may correspond to different triterpenes. The presence of these peaks suggests the existence of compounds with different polarities: according to their retention times, the major ones correspond to more polar compounds, while the minor ones have a lower polarity.

The differences observed in the chemical profiles between the samples from *Aguascalientes* and the galphimins-producing samples from *Dr. Mora*, *Guanajuato*, are possibly related to genetic variations and environmental conditions specific to each population, such as soil characteristics, temperature, moisture, and solar radiation (Balderas et al., 2020; Cardoso-Taketa et al., 2008; Sharma et al., 2012a).

In conclusion, the results obtained by HPLC, complemented by Thin-Layer Chromatography (TLC) analysis, indicate that ethanolic extracts of *Galphimia* spp. populations of *Aguascalientes* do not contain galphimins.

Conclusions

The results of this research suggest that the natural populations of *Galphimia* spp. in *Aguascalientes* are found in degraded environments and, due to their homogeneous phenological and morphological characteristics, can be attributed to the same species.

The HPLC study confirms the absence of galphimins and the analyses by TLC confirm the lack of galphimidins in the populations of *Galphimia* spp. in *Aguascalientes*. The four populations from *Aguascalientes* analyzed by TLC show low-polarity violet metabolites similar to galphimins. These compounds could serve as phytochemical markers for the populations of *Aguascalientes*.

There is a clear need for further biological, chemical and pharmacological research on wild populations of *Galphimia* spp. in *Aguascalientes*. These studies will allow a better understanding of their biological properties, as well as their potential pharmacological, sedative and anti-inflammatory effects, characteristics that have been documented for triterpenes in populations of *Galphimia* spp. collected in other regions of Mexico.

The present study represents a significant advance since it is the first ecological and phytochemical analysis of *Galphimia* spp. in *Aguascalientes*. The results obtained provide phytochemical profiles that confirm the absence of the bioactive compounds described above. However, the detection of other triterpenes suggests the possibility of a unique chemotype in this region, opening new opportunities for future research with advanced techniques such as HPLC-MS and metabolomic analysis. It is also important to further study the phenolic compounds present, given their potential for biological activities of pharmacological and ecological interest.

The results of the present study do not allow to determine whether the material collected in *Aguascalientes* corresponds to *G. glauca*, which should be clarified in future research by means of genetic barcode analysis.

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Conflict of interest

The authors declare that they have no conflict of interest.

Contribution by author

León Arturo Lozano-García: research execution, analysis and interpretation of results and drafting of the manuscript; José de Jesús Luna-Ruiz: research supervision, and analysis and interpretation of results; Joaquín Sosa-Ramírez, Arturo Gerardo Valdivia-Flores, Alexandre Thoshirrico Cardoso-Taketa and María Luisa Villarreal Ortega: project planning and monitoring; Eleazar León-Alvarez and Mónica Morales Aguilar: laboratory analysis and data review.

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