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Metabolic Profiling of the Mexican Anxiolytic and Sedative Plant *Galphimia glauca* Using Nuclear Magnetic Resonance Spectroscopy and Multivariate Data Analysis

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Abstract

Galphimia glauca is popularly employed in Mexico for the treatment of central nervous system disorders. Pharmacological and phytochemical studies have resulted in the identification of the anxiolytic and sedative principle consisting of a mixture of nor-secofriedelanes, named the galphimine series (**1 – 9**). These active constituents were found in plants collected in the vicinity of a restricted region in Central Mexico, where this species is abundant. A metabolic profiling carried out by means of ^{1}H-NMR spectroscopy and multivariate data analysis was applied to crude extracts from wild plant populations, collected from six different locations as a quality control assessment, in order to differentiate their chemical profile. Principal component analysis (PCA) of the ^{1}H-NMR spectra revealed clear variations among the populations, with two populations out of the six studied manifesting differences, when the principal components PC-1 and PC-2 were analyzed. These two PCs permitted the differentiation of the various sample populations, depending on the presence of galphimines. This information consistently correlated with the corresponding HPLC analysis. The neuropharmacological effects of the crude extracts were evaluated by using ICR mice in the elevated plus maze, as well as the sodium pentobarbital-induced hypnosis models. Both assays demonstrated anxiolytic and sedative responses only among those sample populations which had previously been differentiated by PC-1. Partial least square regression-discriminant analysis (PLS-DA) also confirmed a strong correlation between the observed effects and the metabolic profiles of the plants. The overall results of this study confirm the benefits of using metabolic profiling for the *in silico* analysis of active principles in medicinal plants.

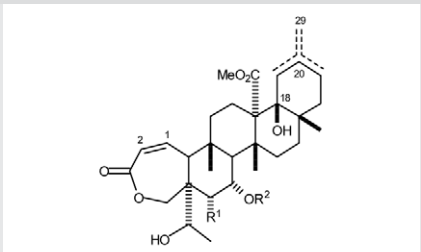
Abbreviations

GM:	Dr. Mora, Guanajuato sample
i.p.:	intraperitoneal
JG:	Guadalajara, Jalisco sample
MC:	Cuernavaca, Morelos sample
MS:	San Andrés de la Cal, Morelos sample
MT:	Tepoztlán, Morelos sample
PC-1:	principal component 1
PC-2:	principal component 2
PCA:	principal component analysis
PLS-DA:	partial least square regression-discriminant analysis
QJ:	Jalpan, Querétaro sample

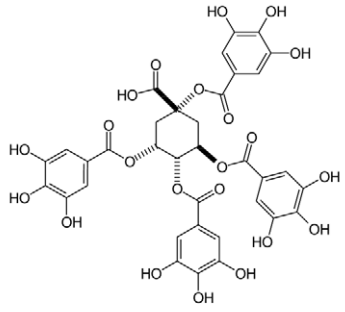
Supporting information available online at http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

The world-wide consumption of phytomedicines has increased significantly over the past two decades, but the lack of accuracy and consistency for defining their composition limits their commercialization. Therefore, it is imperative to implement efficient methods for quality control and standardization of plant active principles. Metabolic profiling provides an important tool for the identification of chemotypes and targets bioactive compounds in herbal drugs [1]. Through an overall analysis of crude extracts, it is possible to discriminate active from non-active plant specimens of the same species found growing in different locations, or exhibiting diverse ontogenetic variables [1], [2].



Galphimine		R^1	R^2
	Δ^{20}		
B	**1**	H	H
E	**2**	OAc	H
A	**3**	OH	H
D	**4**	OH	Ac
	$\Delta^{20(29)}$		
F	**5**	H	H
G	**6**	OAc	H
H	**7**	OH	H
I	**8**	OH	Ac
	Δ^{19}		
C	**9**	H	H



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Galphimia glauca from the Malpighiaceae family provides an appropriate example for metabolic profiling, due to its traditional use in the treatment of central nervous system disorders in Mexico. Previous phytochemical studies resulted in the identification of a mixture of nine nor-secofriedelanes; generically termed as the galphimines (**1 – 9**). These were isolated from the sedative extract, obtained from the aerial parts of the plants [3]. HPLC analysis permitted the resolution of this complex mixture into five peaks. Peaks I, II, IV and V were mixtures of two E-ring double bond isomers, separated by means of argentation preparative-scale thin layer chromatography [3]. NMR spectroscopy analysis identified each isomer as follows: galphimines A (**3**, peak I), B (**1**, peak II), D (**4**, peak IV) and E (**2**, peak V) as pertaining to the endocyclic Δ^{20} series, and galphimines H (**7**, peak I), F (**5**, peak II), I (**8**, peak IV) and G (**6**, peak V) for the exocyclic $\Delta^{20(29)}$ series. Peak III yielded galphimine C (**9**), the Δ^{19} isomer [3].

The sedative effect of galphimine B was documented using *in vitro* models [4]. The anxiolytic effects of galphimines A and B were demonstrated in mice [5]. A clinical study included 152 patients, all exhibiting symptoms of general anxiety, who were treated with preparations of *G. glauca*, confirming the efficacy of this traditional herbal drug [6]. Therefore, *G. glauca* represents an excellent candidate to be developed as a phytomedicine. However, all commercial products available in the market have yet to undergo analytical procedures for the purpose of quality control, as well as pharmacological evaluations.

Previous investigations with this herbal drug have only been conducted on plants growing in a restricted area in the vicinity of the town Dr. Mora, in the state of Guanajuato. However, it remains uncertain whether this chemical profile is shared by specimens growing in other geographical areas outside its natural range, where this herbal drug is being propagated and then commercialized and even exported. The possible chemical and biological variations of this species, when produced in different areas, have never before been studied. In this investigation, a metabolic profiling was carried out, using ^{1}H-NMR spectroscopy and multivariate data analysis techniques [1], [2], employing crude extracts obtained from specimens growing in six different locations in Mexico. This procedure correlated the active extracts and the metabolites responsible for the anxiolytic and sedative properties of this herbal drug.

Materials and Methods

General experimental procedures

^{1}H-NMR experiments (500 MHz) were conducted on a Bruker DMX-500 instrument. The instruments used for the HPLC analysis consisted of a Waters 600E multisolvent delivery system, equipped with a Waters W996 diode array detector (216 nm), and a Waters 717 plus autosampler. Control of the equipment, data acquisition, processing, and management of the chromatographic information were carried out using the Millennium 32 software program (Waters Corporation) PCA and PLS-DA were performed using the SIMCA-P 11.0 software (Umetrics). For the purpose of scaling, Pareto and unit variance methods were applied to PCA and PLS-DA, respectively.

Plant material

Fresh plant material was collected in the summer of 2005 (July and August) from six different locations in Mexico. Voucher specimens were authenticated by Rolando Ramírez and then deposited at the HUMO Herbarium, CEAMISH (Centro de Educación Ambiental e Investigación Sierra de Huautla) UAEM. Collection sample details are presented in **Table 1S** of the Supporting Information section.

Generation of ^{1}H-NMR profiles

A previously reported extraction method using a deutereted protic solvent was employed to obtain a broad range of differing polarity compounds [7]. Pulverized dried leaves (100 mg) of each sample (39) were placed in individual centrifuge microtubes (1.5 mL). CD_3OD (750 μL) was added and the mixture was vortexed (30 s). Then, 750 μL of D_2O with KH_2PO_4 (1.23 % w/w) containing 0.01 % (w/w) TMSP (trimethylsilanepropionic acid sodium salt) was added to each tube. After vortexing (30 s) and sonicating (10 min), the mixtures were centrifuged at 3000 rpm (10 min). The supernatants were transferred to new microtubes (1.5 mL) and recentrifuged (5 min). From each of the extracted solutions, 800 μL were transferred to individual NMR tubes (5 mm) for analysis. The pH of D_2O was adjusted to 6.0 for the NMR measurements with the addition of a 1 M NaOD solution.

For each sample, 128 scans were recorded with the following acquisition parameters: 0.126 Hz/point, pulse width = 4.0 μs (30 °), and relaxation delay = 2.0 s. FIDs were Fourier transformed with line broadening = 0.3 Hz. The spectra were referenced to TMSP at 0.01 % w/v at 0.00 ppm. The regions of δ = 4.52 – 5.08 were excluded from the analysis because of the residual water signals. The ^{1}H-NMR spectra were automatically reduced to ASCII files using AMIX (v. 3.8, Bruker Biospin). Spectral intensities were integrated into regions of equal width (0.02 ppm) between the region of

δ = 0.32 – 10.00. All ^{1}H-NMR signals were normalized to total intensity.

Generation of HPLC profiles

Pulverized dried plant material (100 mg) from each specimen, which included a variety of plant parts (leaves, flowers, stems and roots) was mixed with 500 μL MeOH. The mixture was agitated, sonicated for 10 min and then centrifuged at 3000 rpm for 5 min. The supernatant was removed and the material residue was reprocessed to complete a series of six consecutive extractions. All six samples were combined, evaporated to a volume of 500 μL, and directly injected (10 μL) into the HPLC.

Quantification studies were performed using a Symmetry C_{18} column (Waters; 5 μm, 4.6 × 250 mm), an isocratic elution with CH_3CN-H_2O (1 : 1), a flow rate of 0.7 mL/min, and 10 μL of the sample injection (3 mg/mL). Calibration curves were measured using pure galphimine B (**1**) obtained as previously described [3], to prepare five concentrations within a range of 31.2 – 500 μg/mL for a linear response (R^2 = 0.999). Triplicate evaluations were carried out for each dilution point. The signal-to-noise (S/N) ratio for the standard compound, as described by Snyder [8], was used to establish the limit of detection (LOD S/N ratio = 3) and quantification (LOQ S/N ratio = 10) and was defined as 0.24 and 0.49 μg/mL, respectively.

Neuropharmacological assays

Pulverized dried leaves were extracted with MeOH under sonication for 10 minutes. After filtration and evaporation, crude extracts were subjected to biological assays. Experiments conducted in mice were performed according to the laws and institutional guidelines, and approved by the Ethics and Security Committee from Centro de Investigación en Biotecnología, UAEM. Male ICR mice (33 – 37 g) were housed in groups of 5 per cage, for at least 1 week prior to the experiments. Food and water were available *ad libitum* and the animals were conditioned to a 12 h light/dark cycle, at a temperature of 22 ± 1 °C. Animals were arbitrarily assigned to one of the following lots: group 1: negative controls which received intraperitoneally (*i.p.*) 0.75 mL/100 g of the vehicle (8% Tween 80; Sigma, in saline); group 2: positive controls which received *i.p.* 0.1 mg/100 g of diazepam (Relazepam from Pisa Lab.); groups 3 – 8: the treated individuals each received *i.p.* 50 mg/100 g of crude extracts samples from one of the six plant populations originally collected, dissolved in the same vehicle which was used for the controls. Following their injection, all tested animals were evaluated according to two neuropharmacological models; the elevated plus maze [9] and the sodium pentobarbital-induced hypnosis [10].

Elevated plus maze test. The aluminum platforms of the elevated plus maze were painted white with impermeable epoxy resin. The model consisted of two flat opposing open arms (10 cm wide and 50 cm long) and two opposite enclosed arms, perpendicular to the first pair and of the same size, but with 20 cm high plexiglass enclosing walls. The junction area of the four arms (central platform, 10 × 10 cm) intersected to form the shape of a plus sign. The walls were easily removed for cleaning after each animal was tested. The whole apparatus was elevated to approximately 50 cm above the floor. The testing room was quiet and dimly lit. Mice were individually placed on the open arms, facing the center of the maze. After administration of the test sample (25 min), the activity of the mouse was recorded in relation to the time spent in the enclosed or open arm platforms. The scores were evaluated by applying the formula [open/(open + enclosed)] × 100.

Sodium pentobarbital-induced sleeping time test. Sodium pentobarbital (40 mg/kg) was *i.p.* injected into each mouse, 30 min after the administration of vehicle, diazepam and crude extract samples. Sleeping time was determined as the interval between onset, up until the loss of righting reflex and its recovery. Results were scored in relation to the number of observations for vehicle (11), diazepam (8), GM (13), MT (8), MS (11), MC (11), JG (9), and QJ (13) in both pharmacological tests. Each group consisted of ≥8 mice.

Statistical analysis

SAS (SAS Institute Inc.) 9.1 program, was used for statistical analysis. The results are presented as mean ± S.D. Data were analyzed using a one-way ANOVA, followed by Dunnetts *t*-test. The significant difference was established among groups where the *p* value was lower than 0.05.

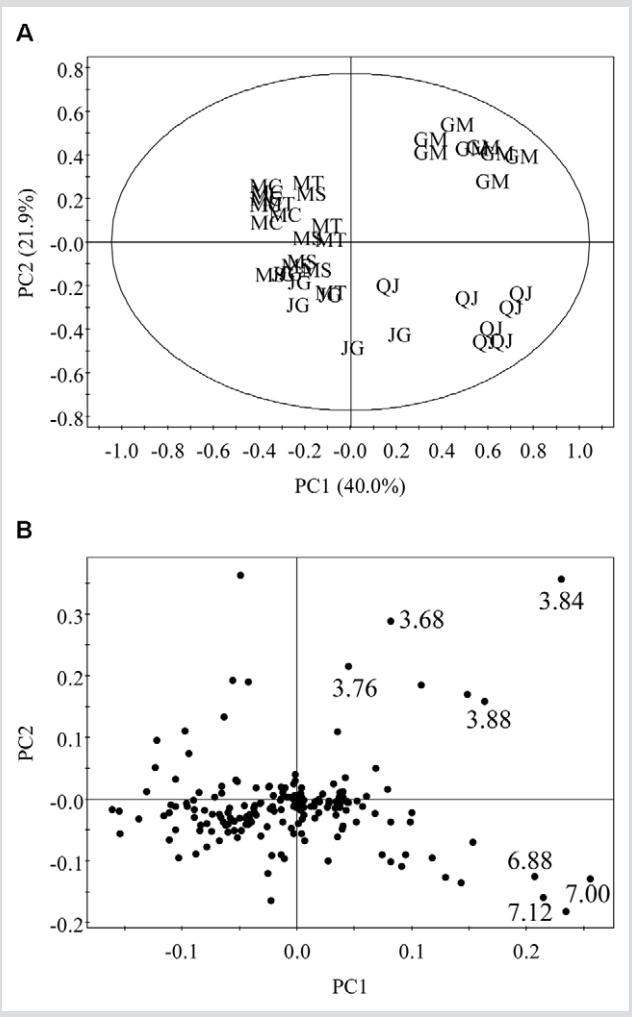

Fig. 1 Score (**A**) and loading (**B**) plots of principal component analysis (PC-1 vs. PC-2) of crude extracts from Dr. Mora, Guanajuato (GM sample); Tepoztlán, Morelos (MT sample); San Andrés de la Cal, Morelos (MS sample); Cuernavaca, Morelos (MC sample); Guadalajara, Jalisco (JG sample) and Jalpan, Querétaro (QJ sample). The ellipse represents the Hotelling T2 with 95% confidence in score plots. Replicates represent separate extractions from individual plants (39 in total).

Results and Discussion

Principal component analysis (PCA) of all ^{1}H-NMR spectra showed a clear difference between the samples harvested in the six locations (**Fig. 1A**), in terms of their galphimines and 1,3,4,5-tetra-*O*-galloylquinic acid contents, thus confirming that these compounds are crucial for differentiation (**Fig. 1B**). The score plot (PC-1 vs. PC-2) in **Fig. 1A** shows that PC-1 and PC-2 clearly separate GM and QJ samples from other plant populations. The loading plot obtained from PCA for all ^{1}H-NMR spectra (**Fig. 1B**) indicated that the signals for the galphimine C-27 methyl ester protons, centered at $\delta = 3.68 - 3.88$, were the main diagnostic resonances for PCA separation. For confirming the presence of galphimines in GM and QJ samples, visual inspection of ^{1}H-NMR spectra was carried out. **Fig. 2** compares the ^{1}H-NMR spectra of the vinylic region for an HPLC peak containing the diastereomeric mixture of galphimines B (**1**) and F (**5**) with that registered for a MeOH crude extract from a GM and a JG samples, with and without galphimines, respectively. The signals H-1 ($\delta = 6.55 - 6.63$) and H-2 ($\delta = 6.00 - 6.05$), which identify the galphimine series [3], were only identified in the GM and QJ spectra, and their presence was corroborated with reference to the cross-peaks which could be observed in their COSY spectra.

In addition to the signals for the galphimine series, the resonances at $\delta = 6.88$, 7.00 and 7.12 were found to be highly important for discrimination of the GM and QJ samples which further allowed their separation. They were assigned to the galloyl moieties of 1,3,4,5-tetra-*O*-galloylquinic acid, a metabolite previously reported as being present in this plant material [11]. The loading plot (**Fig. 1B**) showed that the resonances of galloyl moieties of 1,3,4,5-tetra-*O*-galloylquinic acid were another main feature indicating PCA discrimination. QJ samples were found to contain a higher level of 1,3,4,5-tetra-*O*-galloylquinic acid than GM samples. As minor contributions H-6 (δ 6 .24, d, $J = 2.0$ Hz) and H-8 ($\delta = 6.40$ d, $J = 2.0$ Hz) of quercetin analogues were found to be more accumulated in GM and QJ samples. However, the H-8 of phenylpropanoids at $\delta = 6.36$ (d, $J = 16.0$ Hz) [12] showed higher levels in MT, MC and JG samples.

This metabolic profiling based on ^{1}H-NMR spectra and PCA data suggests that the chemical differentiation may have direct impact on the pharmacological effects, and for this reason ethnomedical studies have often recorded varied uses for the same plant in communities separated even by short distances. These results also give reason for the traditional selectivity employed in the preparation of this plant material for herbal remedies.

For the evaluation of the anxiolytic and sedative activity of crude extracts, the neuropharmacological effects were evaluated by using ICR mice in the elevated plus maze, as well as in the sodium pentobarbital-induced hypnosis models.

The elevated plus model is designed to determine rodent anxiety levels in a new exploratory environment, with the expectation that untreated animals will spend more time in the enclosed arms than in the less secure open arms. This model is one of the most validated tests for assaying anxiolytic substances [13]. The results in **Fig. 3A** show a significant difference between animals injected with vehicle and those injected with diazepam (vehicle 18.0 ± 8.6%; diazepam 56.5 ± 11.2%). Of those injected with the crude extracts samples, only the mice treated with GM and QJ spent a higher percentage of time in the open arms [GM 38.0 ± 11.4%; MT 28.0 ± 11.2%; MS 22.8 ± 9.3%; MC 28.5 ± 7.3%; JG 27.1 ± 13.6%; QJ 36.8 ± 7.6%. $F(2.13) = 12.94$, $p < 0.05$].

The sodium pentobarbital-induced hypnosis model measures the latency and duration of sleeping time in mice exposed to sedative compounds. **Fig. 3B** shows that GM, QJ and MT crude extract samples manifested significant differences in terms of their levels of sedation, when the sodium pentobarbital-induced hypnosis test was applied [vehicle 44.8 ± 6.9%; diazepam 74.1 ± 1.8%; GM 68.1 ± 8.1%; MT 54.1 ± 5.7%; MS 51.3 ± 4.1%; MC 50.1 ± 11.3%; JG 50.8 ± 6.0%; QJ 66.5 ± 8.4%. $F(2.13) = 21.17$,

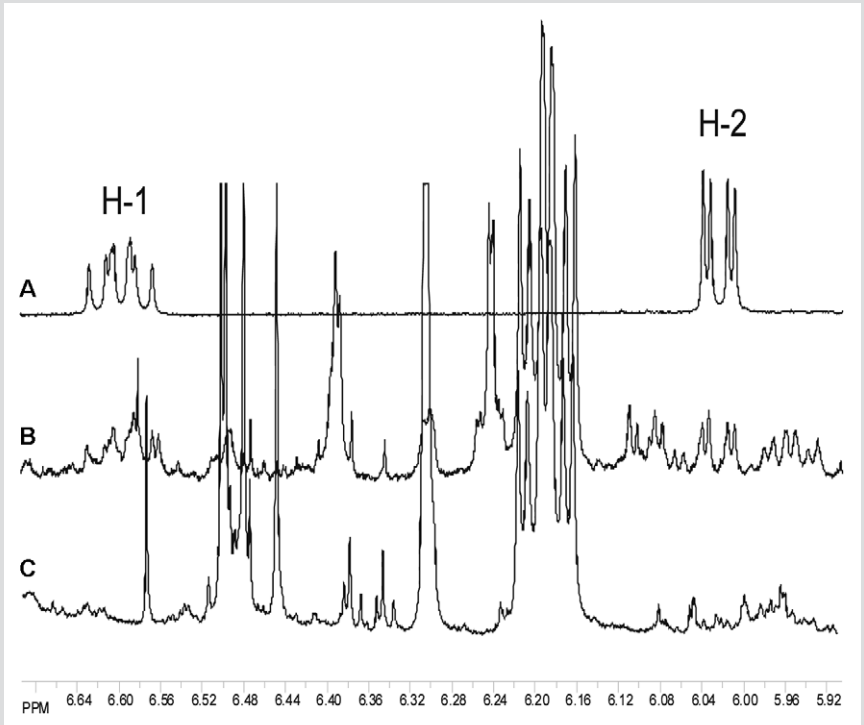

Fig. 2 ^{1}H-NMR spectra of: **A**) resonances for vinylic protons H-1 and H-2 of an HPLC peak containing the diastereomeric pair of galphimines B (**1**) and F (**5**); **B**) an MeOH crude extract from one individual collected at Dr. Mora, Guanajuato (GM sample) showing the resonances for H-1 ($\delta = 6.55 - 6.63$) and H-2 ($\delta = 6.00 - 6.05$) for the galphimine series and **C**) a MeOH crude extract from one individual collected at Guadalajara, Jalisco (JG sample) without galphimines.

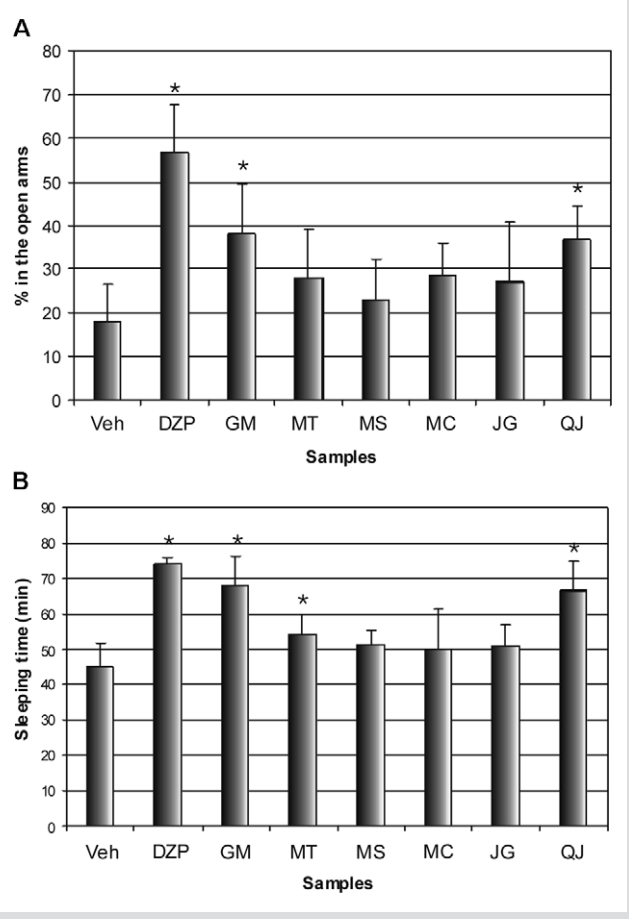

Fig. 3 Effect of administration of 50 mg/100 g *G. glauca* crude extracts (GM, MT, MS, MC, JG and QJ), control vehicle (0.75 mL/100 g at 8% Tween 80 in saline) and diazepam (0.1 mg/100 g) in two neuropharmacological models: **A**) elevated plus maze evaluating the percentage of time spend in the open arms (after twenty five minutes *i. p.* injection), and **B**) sodium pentobarbital-induced hypnosis (after thirty minutes *i. p.* injection), on the sleeping time measured as the time spent between disappearance and reappearance of the righting reflex. * $p < 0.05$ on the ANOVA followed by *post hoc* Dunnett's *t*-test (mean ± S.D.).

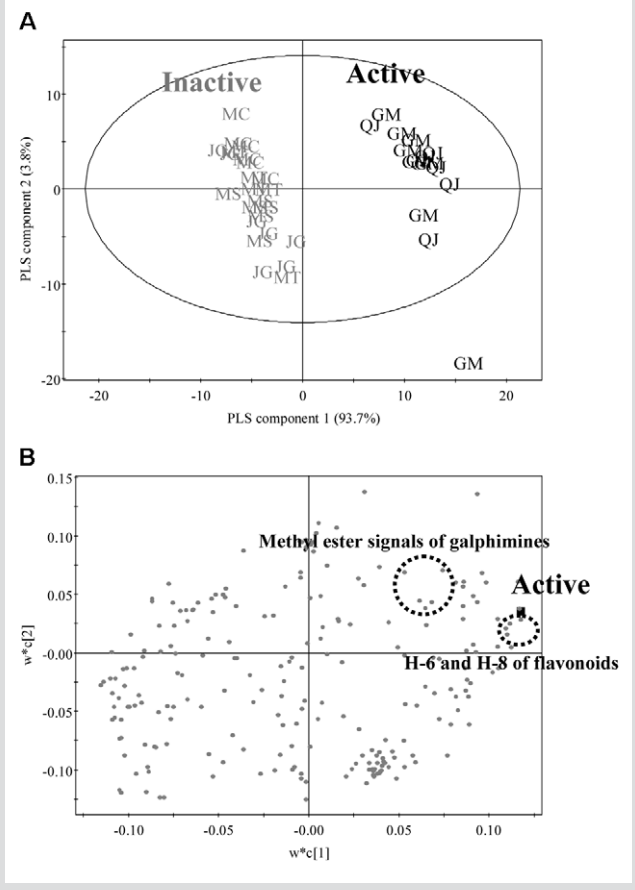

Fig. 4 Score (**A**) and loading (**B**) plots of PLS-DA models (PLS component 1 vs. PLS component 2) based on ^{1}H-NMR resonances and anxiolytic activity of *G. glauca* crude extracts based on two classes, active and inactive plants. Active (> 36.7%) plant materials include GM and QJ. Inactive plant materials (< 28.6%) include MT, MS, MC and JG.

$p < 0.05$]. In both neuropharmacological tests, the plants containing higher level of galphimines such as GM and QJ showed better activity. These results may indicate that the crude extracts containing galphimines were responsible for the anxyolytic effect, traditionally attributed to this herbal drug.

In order to prove direct mutual correlation between metabolic profiling data and bioactivity (anxiolytic and sedative activities), a supervised multivariate data analysis method using covariance between two datasets, e. g., metabolic profiling and bioactivity data [14], was required. Although PCA is one of the most popular unsupervised multivariate data analyses for data reduction in the field of metabolic profiling, the separation is achieved only by the variation of one dataset (^{1}H-NMR data in the present work). In this study, partial least square regression modeling-discriminant analysis (PLS-DA) was used. A discrete class matrix (0 and 1), as additional Y-matrix, was applied in order to correlate metabolic profiling with two tested neuropharmacological activities. Based on the activity in the elevated plus model experiment, the samples were divided by two classes, active (> 36.7%) and inactive (< 28.7%) for PLS-DA. In the case of sodium pentobarbital-induced hypnosis model, two classes including active (> 51.4 min) and inactive (< 51.3 min) were employed.

In the results of PLS-DA, galphimine was found to be clearly associated with the neuropharmacological activities evaluated in this study (**Fig. 4** and **Fig. 5**). In addition to these triterpenoids, quercetin analogues were also found to be responsible for discrimination, and their contribution to the neuropharmacological activities remains to be determined experimentally. In the case of MT samples, they showed a minor sedative effect even though galphimines were absent (**Fig. 5**). It suggests that other compounds may affect the sedative activity.

Variance (R^2) and cross-validated variance (predictive ability of the model, Q^2) values of PLS-DA using two components were calculated (a value of $Q^2 > 0.5$ is generally accepted to be good) [14]. For elevated plus maze activity the figures were 0.98 and 0.96, respectively; for the sodium pentobarbital-induced hypnosis activity the values were 0.77 and 0.64, respectively. Both PLS-DA models were validated by the permutation method through 20 applications in which all Q^2 values of permuted Y vectors were lower than original ones and the regression of Q^2 lines intersect at below zero (**Fig. 6**).

The identification and quantification of galphimines A - I (**1 – 9**) were carried out by means of reverse-phased HPLC, by comparing peak retention time and coelution experiments on authentic

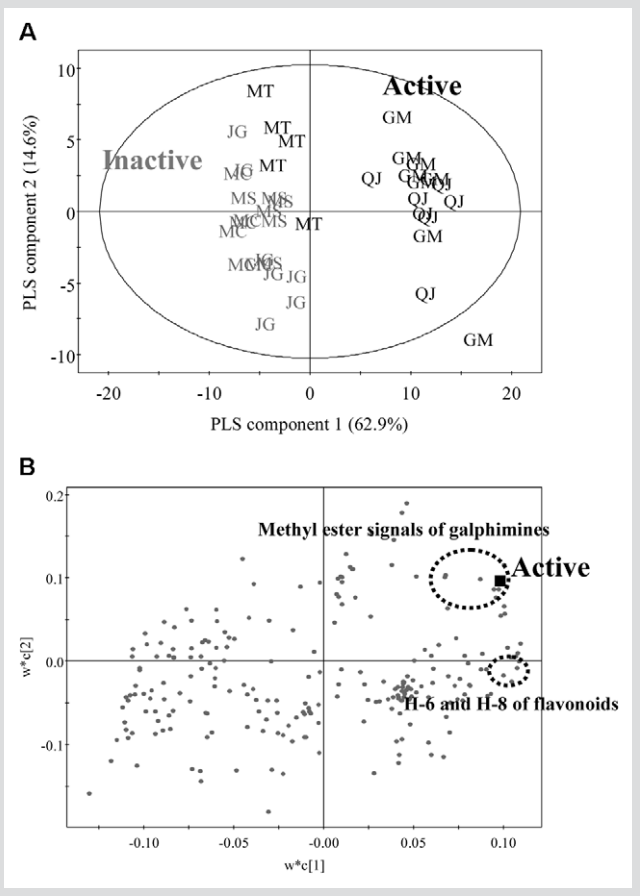

Fig. 5 Score (**A**) and loading (**B**) plots of PLS-DA models (PLS component 1 vs. PLS component 2) based on ^{1}H-NMR resonances and sedative activity of *G. glauca* crude extracts based on two classes, active and inactive plants. Active (>51.4 min) plant materials include GM, MT, and QJ. Inactive (<51.3 min) plant materials include MS, MC and JG.

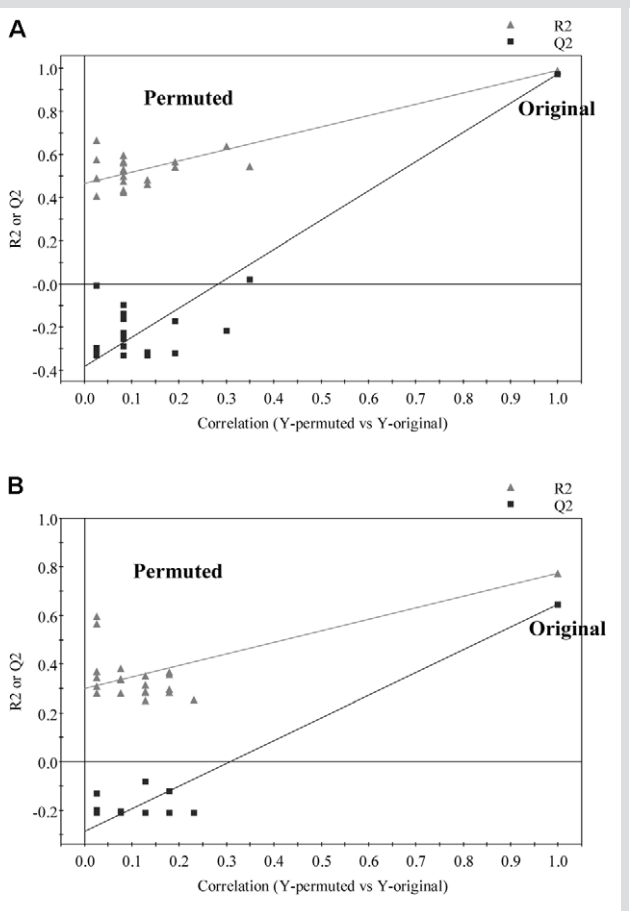

Fig. 6 Validation plot of PLS-DA models using ^{1}H-NMR resonances and anxiolytic activity (**A**), and sedative activity (**B**) of *G. glauca* crude extracts based on two classes.

samples (▶ **Fig. 7**). The chromatographic peaks for these nor-secofriedelanes were only detected for the GM and QJ samples, whilst none were discovered in the four other locations. These results agreed with those of PCA. The highest total accumulation (6.58 mg/g DW and 5.66 mg/g DW, respectively) was recorded for leaf samples and was not statistically different for both populations, whereas for flowers these values were 2.58 and 2.52 mg/g DW, respectively. Traces of these triterpenes were identified in the stems, but not in the roots. ▶ **Table 1** shows the accumulated galphimine values for each of the individually tested GM and QJ samples, revealing that galphimines B (**1**) and F (**5**) generally represented the highest content. As these results have indicated, there may be drastic differences in terms of the amount of bioactive glaphimines in samples, depending on the location from which they are collected.

^{1}H-NMR spectroscopy of all 39 crude extracts permitted the generation of metabolic profiles, useful for observing a wide range of secondary metabolites and for detecting target compounds by means of clear diagnostic signals such as galphimine and 1,3,4,5-tetra-*O*-galloylquinic acid. PLS-DA further clarified the distinctions used for classifying the GM and QJ samples, in terms of whether they constitute sedative and anxiolytic plant material, thus highlighting the effectiveness of this predictive multivariate data analysis model for *in silico* analysis. This is the first time that a metabolic profiling analysis, using NMR and multi-

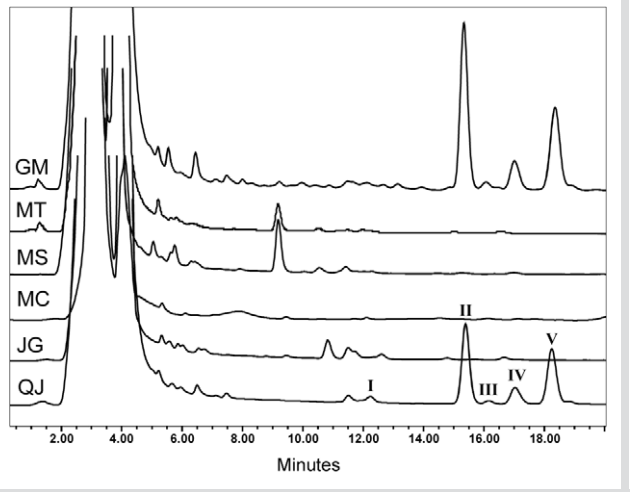

Fig. 7 Reversed phase HPLC separations of the sedative triterpenes crude extracts of *G. glauca* obtained from six different locations GM, MT, MS, MC, JG and QJ. Peak assignments: I, compounds **3** and **7** (t_R = 12.25 min); II, compounds **1** and **5** (t_R = 15.41 min); III, compound **9** (t_R = 16.18 min); IV, compounds **4** and **8** (t_R = 17.05 min); peak V, compounds **2** and **6** (t_R = 18.30 min).

Table 1 Accumulation of the nor-secofriedelane galphimines (mg/g DW) in leaves obtained from *G. glauca* individuals growing in Dr. Mora, Guanajuato (GM samples) and Jalpan de Serra, Querétaro (QJ samples)

Sample (leaves)	Peak I (GA + GH)	Peak II (GB + GF)	Peak III (GC)	Peak IV (GD + GI)	Peak V (GE + GG)	Total Galphimines
GM_1	0.12	3.32	0.08	1.09	3.18	7.79
GM_2	0.24	3.24	0.10	0.74	2.32	6.65
GM_3	0.04	2.74	0.08	0.63	1.89	5.38
GM_4	0.08	3.61	0.11	0.96	2.80	7.56
GM_5	0.04	3.24	0.09	0.63	1.95	5.95
GM_6	0.07	3.02	0.08	0.72	2.27	6.16
GM_7	0.08	3.22	0.08	0.81	2.50	6.69
GM_8	0.11	3.08	0.09	0.80	2.35	6.43
Mean	**0.10**	**3.18**	**0.09**	**0.80**	**2.41**	**6.58** *
QJ_1	0.10	2.28	0.07	0.59	1.75	4.79
QJ_2	0.25	2.13	0.08	0.81	1.96	5.23
QJ_3	0.19	2.50	0.07	0.67	2.07	5.50
QJ_4	0.05	3.45	0.09	0.26	0.81	4.66
QJ_5	6.20	4.00	0.10	–	–	10.3
QJ_6	0.27	2.20	0.08	0.48	1.62	4.65
QJ_7	0.87	1.99	0.05	0.41	1.19	4.51
Mean	**1.13**	**2.65**	**0.08**	**0.46**	**1.34**	**5.66** *

* $p < 0.05$ following Tukey analysis.

variate data analysis, was applied to a Mexican herbal drug for the purpose of differentiating populations according to their bioactive principle content, and then to correlate these finding with their observed pharmacological efficacy. The information obtained in this work may be used in the future to establish the analytical requirements for standardization of quality control processes for phytomedicines based on this herbal drug.

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