Pastora Salinas-Hernández[1]
Francisco J. López-Bermúdez[1]
Damaris Rodríguez-Barrientos[2]
María Teresa Ramírez-Silva[2]
Mario A. Romero-Romo[3]
Fernando Morales-Anzures[1]
Alberto Rojas-Hernández[2]

[1]Instituto de Estudios, Universidad del Istmo Campus Tehuantepec, Av. Universidad S/N, Oaxaca, México
[2]Departamento de Química, Universidad Autónoma Metropolitana-Iztapalapa, México, D.F. México
[3]Departamento de Materiales, Grupo de Ingeniería de Materiales, Universidad Autónoma Metropolitana-Azcapotzalco, México, D.F. México

Original Paper

Development of a capillary electrophoresis method for the characterization of "palo azul" (*Eysenhardtia polystachya*)

The tree *Eysenhardtia polystachya* (Ortega) Sarg. has quite a wide popular use within the traditional Mexican medicine as herbal remedy. Popular practices constitute a relevant enough basis to design optimum analytical methods in order to determine basic principles of diverse medicinal plants. This has become one of the essentials needed to characterize such products, for which it is fundamentally important to develop an efficient and reliable separation method. This work presents the results concerning the development and optimization of a novel CE method for the separation of components from water/etanol (1:1) extracts of *E. polystachya*, using the following conditions, considered the best obtained: phosphate buffer 10 mM, 20 kV voltage, and pH 8.1 at 214 nm and 50 mM, 12.5 kV voltage with pH 8.1 at 426 nm. The optimization takes into account the parameters associated in the resulting electropherograms, such as number of peaks, migration times, and the Δt_m of the neighboring peaks. Under optimal conditions the separation intended was attained within 15 and 20 min for 214 and 426 nm, respectively. The characterization method developed was applied to the analysis of diverse extracts of *E. polystachya*.

Keywords: Characterization method / CZE / *Eysenhardtia polystachya* / Fingerprints / Mexican "palo azul"

Received: August 2, 2007; revised: November 23, 2007; accepted: November 24, 2007

DOI 10.1002/jssc.200700368

1 Introduction

Presently, natural medicine constitutes a valid option closely associated to the need to achieve further progress on predictive and preventive measures to strengthen world public health. Recently, the World Health Organization has estimated that herbalism is recognized as three or four times as widespread compared to traditional medicine. Further, it has been considered that half of the modern medicaments come from the vegetal kingdom [1], which is indeed an incontrovertible fact.

On consideration of the aforementioned, optimal analytic methods are thus required to characterize and determine basic components of medicinal plants in order to proceed to viable extraction and separation of diverse basic principles for the manufacture of new pharmaceuticals, which as a matter of course, is a growing need.

Correspondence: Dr. Alberto Rojas-Hernández, Departamento de Química, Universidad Autónoma Metropolitana-Iztapalapa, Área de Química Analítica, San Rafael Atlixco 186, Col. Vicentina, 09340 México, D.F. México
E-mail: suemi918@xanum.uam.mx
Fax: +52-55-58044666

The tree known as *Eysenhardtia polystachya* belonging to the Leguminosae family, is fairly small and is mostly distributed in a large region of the American continent, from the southern part of Arizona state in the United States, down to Oaxaca state in the southwestern part of México [2]. Commonly known in Spanish as *palo azul* (blue wood), is also known as *tlapalezpatli* in Nahuatl dialect, *urza* in Otomi dialect, the latter two being other names given in various localities [3], has widespread use in the traditional Mexican medicine as herbal remedy [4]. The plant has been used in traditional treatments of nephrolithiasis or urolithiasis [2], lumbalgia, arthritis, rheumatism, sciatica, and as blood depurative because it eliminates uric acid, of which application the *palo azul* derives its diuretic and antirheumatic fame. For ingestion purposes, the wood chips are brought to boil in water to produce a golden-colored liquor having a light blue fluorescence, from which the sick can take two or three cups a day [3].

Among the *E. polystachya* components, there can be found polyphenol compounds, tannins, or flavonoid compounds [5, 6], which are a variable and complex mixture of bitter, astringent chemicals, although in general these are esters from one kind of sugar having a variable number of phenol acids [7].

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

The *E. polystachya* has been the subject of diverse physicochemical studies [8–10], in part because the *Eysenhardtia* species is more widely used for medicinal purposes [11]. The chemical studies on this species have facilitated the isolation of several isoflavones [5–10], the separation of which from the remainder of the constituents is done through silica gel chromatographic column, repeating the procedure several times. Thus, this is a lengthy, expensive method that uses considerable amounts of solvent. The structures of the compounds were identified through HPLC and spectroscopic methods such as ^{1}H and ^{13}C NMR.

The CE is basically a separation technique based on the differential mobility or migration rate of charged particles under the influence of an electric field. The CE is a fairly recent technique surprisingly not much used yet to characterize herbal extracts [12, 13], although CE has basic advantages over other traditional separation techniques, including HPLC, because among other things, it requires quite a small amount of sample and small solute concentrations; apart from the fact that is very simple because it just measures migration time, it does not require treatment compounds having high purity. The development of a rapid, efficient, and selective separation method requires optimization of the separation conditions; once the optimized parameters are obtained, the application of the method is rapidly and easily done to characterize samples by means of the CE fingerprints. Therefore, this work aims to propose a CE method to exert the separation of the extracts of the *E. polystachya*.

2 Experimental

2.1 Reagents

The wood of the *E. polystachya* tree used was obtained from the Laboratory of Herbal Remedies, Fabrication of Fluid and Dried Extracts, Rosa Elena Dueñas (LHRFFDE), and also from the plants market of Oaxaca, Mexico. Extracts were prepared using ethanol/water (1:1), acetone/water (70:30), and water as extracting agents, suspending 5 g of the wood chips and 100 mL of each solvent at reflux temperature; thereafter it was cooled, filtered, and stored in amber bottles at 4°C.

All substances used through the studies herein reported were of analytical grade. Acetone, ethanol, and HCl were supplied by J. T. Baker; the extra pure grade tannic acid and the hydrated epicatechin, catechin, and floretin were supplied by Aldrich. The borate buffer containing 98.3% water, 0.4 boric acid, and 0.3 sodium borate, was supplied by Beckman Coulter. The phosphate buffer was made from Na_2HPO_4 0.5 M solution and NaH_2PO_4 0.5 M solution, both from Fluka 99% purity. All solutions were prepared with deionized water, with a resistivity of 18 MΩ cm, free from organic matter.

2.2 Equipment and CE

The CE experiments were performed with the aid of a P/ACE MDQ CE System (Beckman Coulter) equipped with a photodiode array detector (PAD) module from 190 to 600 nm, controlled through the 32 Karat software in an IBM PC with Pentium 4. The fused-silica capillary tubes were supplied by Beckman Coulter with 60.2 cm overall length (50 cm to the detector) and 50 μm id. The pH was determined by means of a Mettler-Toledo MP230 pH-meter.

The detection was performed on the cathodic side, applying a normal polarity voltage. Borate and phosphate buffers were used. The capillary was cleansed daily during 5 min with NaOH 0.1 M, 5 min with H_2O and 5 min with buffer. In between each run, the capillary was also conditioned for 1 min with NaOH, 1 min with H_2O at 25°C, 1.5 min with the borate or the phosphates buffer, and at the end of the day, the capillary was washed with NaOH 0.1 M and with H_2O for 5 min, leaving the capillary filled with water.

2.3 Electrophoretic analysis

For the electrophoretic analysis both buffers, borates and phosphates, were used to determine which gave the best resolution. In order to obtain the best electropherograms of the extract, a silica column was used with a pH 8.1 imposed through either the borates or phosphates buffer, monitoring the UV–Vis spectra in the range of 190–600 nm.

2.4 Optimization parameters and repeatability

As the *E. polystachya* extracts exhibit a red color, the analysis were performed in the UV (214 nm) and in the visible (426 nm) regions, for which the optimization parameters were determined on each. For each zone of interest the voltage as well as the buffer concentration was varied.

For the analysis of the CE, both the phosphate and borate buffers were tested: their concentrations varied from 10 to 50 mM and the voltage also varied in the range of 10–25 kV. Various peak parameters were assessed, namely, migration time and Δt_m for the neighboring peaks. The resolution was optimized by obtaining the maximum of the difference of the migration time of the nearest peaks *versus* voltage and buffer concentration. Repeatability was evaluated by using one-way ANOVA test of the migration time of the peaks.

3 Results and discussion

A test run was carried out for each of three extracts, namely ethanol/water (1:1), acetone/water (7:3), and water. Further, in order to decide which of the buffers

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

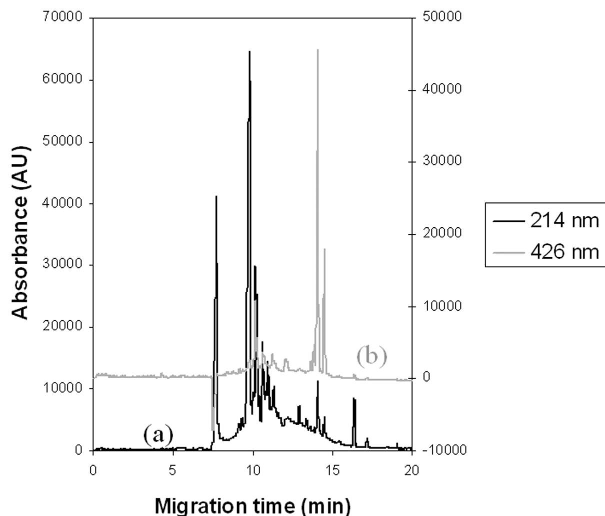

Figure 1. Electropherograms of the extract prepared using 5 g of the vegetal material and 100 mL of ethanol/water (1:1) as extracting agent; with 15 kV, and 50 mM phosphate buffer at pH = 8.1. (a) Electropherogram recorded at 214 nm. (b) Electropherogram recorded at 426 nm.

was going to be employed for the determinations with the best extract, both the borate and phosphates buffers were used; subsequently, the resulting electropherograms from every extract were duly analyzed. The phosphate buffer for the ethanol/water (1:1) extract gave a better CE fingerprint, because it exhibited the largest polyphenol or flavonoid content and the greatest number of peaks; thus these were the extract and buffer to be used further.

The *E. polystachya* extract is a complex mixture of polyphenol compounds [5 – 10], which are difficult to identify satisfactorily through conventional chromatography methods; thus, it is of great importance to obtain optimum parameters for their analysis. Once these parameters were fixed, every analysis became more efficient with respect to the resolution of the corresponding electropherograms.

For the optimization of a CE method, there is a series of parameters that need to be considered such as voltage, pH, and the buffer concentration, which should provide the means for a good separation with a large enough number of peaks having significant migration times differences, Δt_m, with respect to their neighboring peaks; needless to say, the exactness of the experimental measurements, or mobility, is largely dependent on such factors.

The study consisted in finding out the most appropriate concentration for the analysis of the extracts, capable of providing neither very long nor short migration times, which is equivalent to say giving good peak resolution. The electropherograms obtained allowed determination of the optimal parameters for analyzing the extracts. When the electropherograms were being analyzed, it

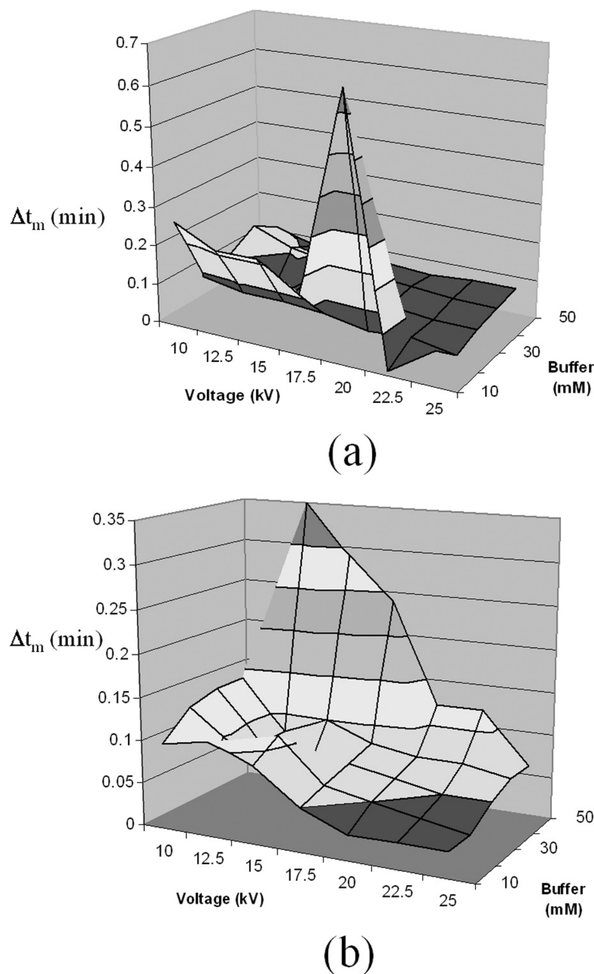

Figure 2. Δt_m of the neighboring peaks of each electropherogram obtained as a function of the buffer concentration and of the voltage for the *E. polystachya* extract. (a) In the UV region at the 214 nm wavelength with 10 mM buffer concentration, voltage 20 kV and pH = 8.1. (b) In the visible region at the 426 nm wavelength with 50 mM buffer concentration, voltage 12.5 kV and pH = 8.1.

was observed that in the 214 nm wavelength of the UV region, there appeared some peaks which were not so easily detected in the 426 nm visible region, or conversely, as it can be seen in Fig. 1.

Optimum conditions were determined for each region of interest, namely, UV and visible at the 214 and 426 nm, respectively, at a pH = 8.1, as it can be seen in Fig. 2. For the former region, the optimum parameters were 10 mM buffer concentration and 20 kV (Fig. 2a). For the latter region, the optimum parameters were 50 mM buffer concentration and 12.5 kV (Fig. 2b).

3.1 Repeatability

Repeatability of the electropherograms was evaluated for the *E. polystachya* extract, for which seven samples of

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

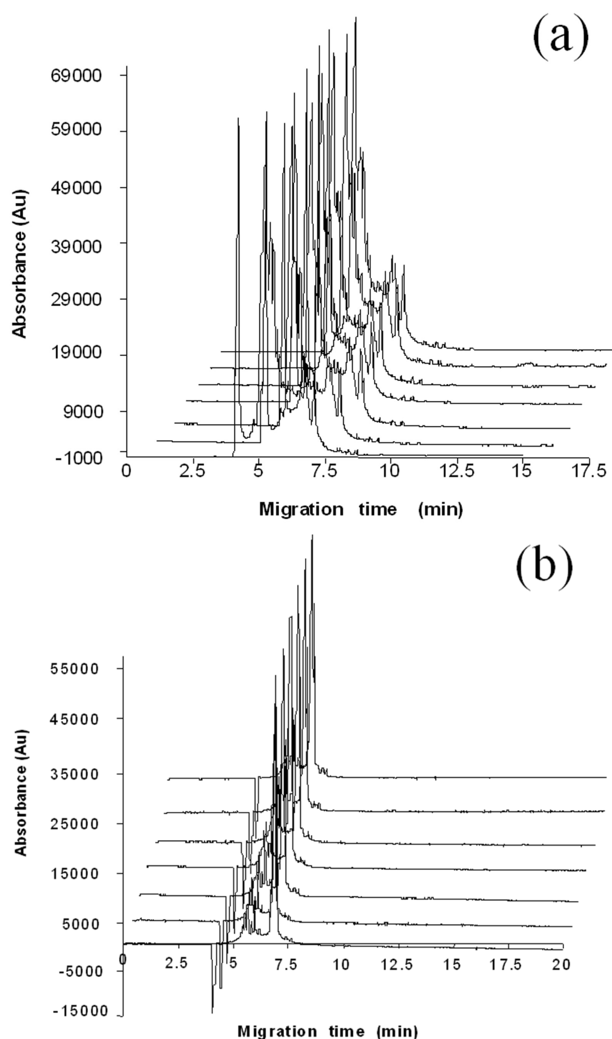

Figure 3. Repeatability of the electrophoretic separation of the analyses of 50 mg/mL of *E. polystachya* extract of LHRFFDE sample under optimum conditions. (a) Electopherograms for the UV region at a wavelength of 214 nm with an RSD of 0.019. (b) Electropherograms for the visible region at a wavelength of 426 nm with an RSD of 0.005.

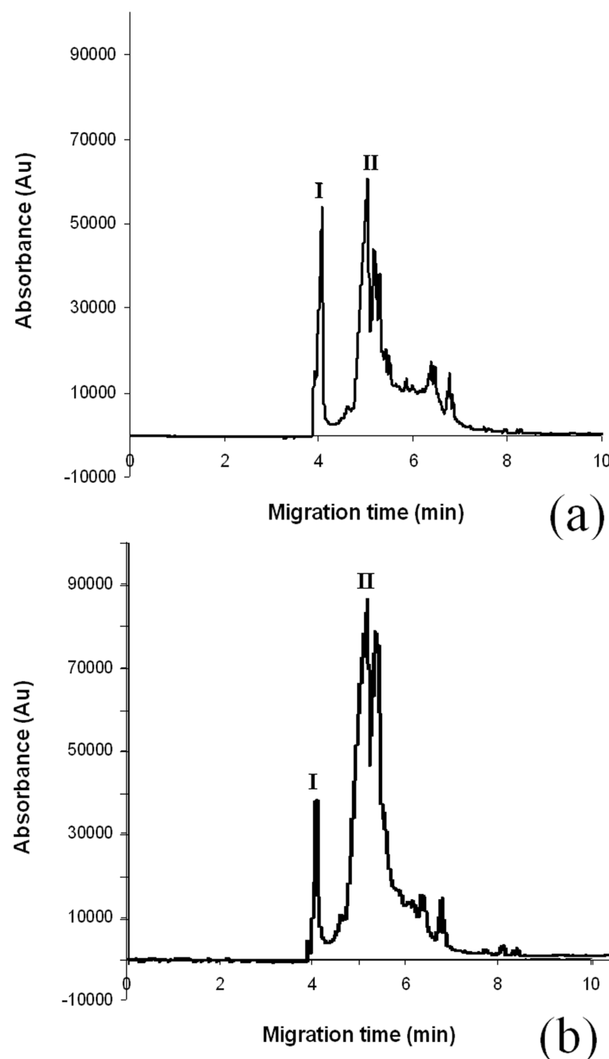

Figure 4. (a) Electropherogram of the 50 mg/mL extract of *E. polystachya* at 214 nm wavelength in the UV region, under optimum conditions: 10 mM phosphate buffer, 20.0 kV, and pH = 8.1, 1 s hydrodynamic injection, at 25°C. (b) Electropherogram of the *E. polystachya* extract supplied by the LHRFFDE under optimum conditions at 214 nm wavelength of the UV region. Peaks I and II may be due to catechins or other flavonoid compounds. Tannic acid could be into the samples at a migration time about 6 min.

each wood were injected under optimum conditions as defined above for each region UV and visible, respectively. Figure 3 shows the results obtained for repeatability of LHRFFDE extract.

One-way ANOVA considering the migration times of all the peaks are shown in Table 1. These results allowed assuring that the method optimized is reproducible for both optimized electropherograms that may be considered as fingerpritns of *E. polystachya* extracts.

3.2 Application

The method developed was applied to the analysis of the commercial wood *E. polystachya*. The extract was obtained using 5 g of vegetal material and 100 mL of the mixture of ethanol/water (1:1) as extracting agent. The extract was cooled, filtered, and stored in amber bottles at 4°C. An aliquot from the extract was taken to obtain a solution with a concentration 50 mg/mL. The electropherograms obtained at 214 or 426 nm displayed a close similarity, except for small shifts in migration times of some of the peaks, but some of them are greater in the first or in the second case, as shown in Figs. 4 and 5. Furthermore, peaks I and II shown in Fig. 4, could be assigned to catechins or other flavonoid compounds (such as floretin), as well as peak III in Fig. 5. This preliminary assignation was obtained by the analysis and comparison of

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim

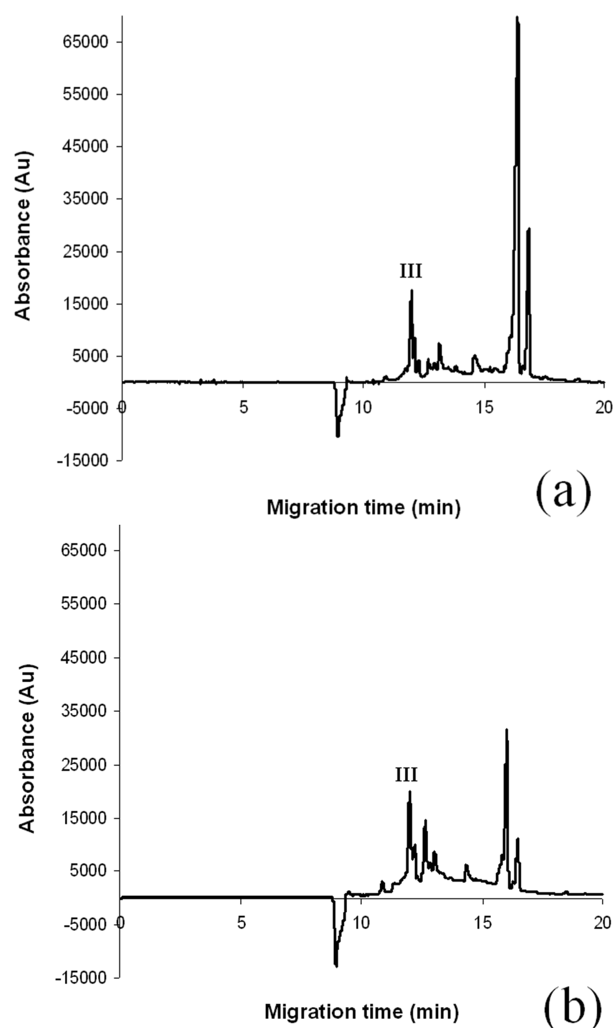

Figure 5. (a) Electropherogram of the 50 mg/mL extract of *E. polystachya* at 426 nm wavelength in the visible region, under optimum conditions: 50 mM phosphate buffer, 12.5 kV, and pH = 8.1, 1 s hydrodynamic injection, at 25°C. (b) Electropherogram of the *E. polystachya* wood supplied by the LHRFFDE under optimum conditions at 426 nm wavelength of the visible region. Peak III may be due to catechins or other flavonoid compounds.

Table 1. Results of one way ANOVA for the migration times of the peaks for electropherograms recorded at 214 and 426 nm

Wavelength (nm)	Freedom degrees	SS	MS	RSD of t_m
214	6	0.263	5.749	0.019
426	6	0.417	15.084	0.005

The separation conditions are the optimal for each case.

migration times of the peaks in corresponding electropherograms of samples with known standards in optimal conditions. There may be some peaks at a migration time of about 6 min in Fig. 4a (optimal conditions for 214 nm) which could be assigned to tannic acid.

Figures 4 and 5 also show that all the corresponding peaks are present at same migration times of the two different samples of *E. polystachya* studied in the present work. The only difference between them is observed in the height and area of the peaks, as expected by concentration of each component in different natural samples of the same plant.

4 Concluding remarks

The method developed in this study was successfully applied to analyze samples of *E. polystachya*, allowing separation of its main constituents of extracts obtained with mixtures of water/etanol (1:1). Nevertheless, we propose to obtain two fingerprints to characterize the samples of *E. polystachya*; these fingerprints are shown in Figs. 4 and 5. The knowledge of these behavior patterns allows identification provided a given extract belongs to this kind of wood and to give a powerful tool to the quality control and normalization of natural extracts. The identification of each one of the peaks of both fingerprints and optimization studies by ED-ANN approach of these and other Mexican herbal extracts will be a matter of further studies.

The authors wish to acknowledge partial financial support to CONACYT through project 46124 and through the Analytical Chemistry (UAM-I) and the Materials Engineering (UAM-A) Research Groups (Cuerpos Académicos) of PROMEP. Also, M. T. R. S., A. R. H., and M. A. R. R. gratefully thank the SNI for the distinction of their membership and the stipend received.

5 References

[1] National Policy on Traditional Medicine and Regulation of Herbal Medicines: *Report of a WHO Global Survey*, World Health Organization, Geneva 2005, p. 5.

[2] Rzedowski, J., *Vegetación de México*, Limusa, Mexico 1978, p. 202.

[3] Álvarez, J. R., *Enciclopedia de México*, Vol. XI, Sabeca Int. Inv. Corp., Enciclopedia Británica de México, Mexico 1994, p. 6167.

[4] Hernández, F., *Historia Natural de la Nueva España*. *Obras Completas de F. Hernández*, Vol. II, UNAM, Mexico 1959, pp. 173 – 212.

[5] Alvarez, L., Rios, M. Y., Esquivel, C., Chávez, M. I., Delgado, G., Aguilar, M. I., Villareal, M. L., Navarro, V., *J. Nat. Prod*. 1998, *61*, 767 – 770.

[6] Alvarez, L., Delgado, G., *Phytochemistry* 1999, *50*, 681 – 687.

[7] Dey, P. M., Harborne, J. B., *Methods in Plant biochemistry. Vol. 1: Plant Phenolics*, Academic Press, New York 1988.

[8] Domínguez, X. A., Franco, R., Díaz Viveros, Y., *Rev. Latinoamer. Quim*. 1978, *9*, 209 – 211.

[9] Beltrami, E., De Bernardi, M., Fronza, G., Mellerio, G., Vidari, G., Vita-Finzi, P., *Phytochemistry* 1982, *21*, 2931 – 2933.

[10] Hastings, R. B., *Econ. Bot*., 1990, *44*, 336.

[11] Burns, D. T., Dalgarno, B. G., Gargan, P. E., Grimshaw, J., *Phytochemistry* 1984, *23*, 167 – 169.

[12] Shaban, E., Dagmar, G., Havel, J., *J. Sep. Sci*. 2006, *29*, 1174 – 1179.

[13] Hameda, A. B., Gajdošová, D., Havel, J., *J. Sep. Sci*. 2006, *29*, 1188 – 1192.

© 2008 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim