Microchemical Journal 112 (2014) 153–158

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Micellar electrokinetic chromatography of the constituents in Philippine *lagundi* (*Vitex negundo*) herbal products

Heide R. Rabanes [a,b,c], Armando M. Guidote Jr. [b], Joselito P. Quirino [a,b,*]

[a] Australian Centre for Research on Separation Science (ACROSS), School of Physical Sciences-Chemistry, University of Tasmania, Hobart, Tasmania, Australia
[b] Department of Chemistry, School of Science and Engineering, Loyola Schools, Ateneo de Manila University, Quezon City, Philippines
[c] Chemistry Department, Xavier University-Ateneo de Cagayan, Cagayan de Oro City, Philippines

ARTICLE INFO

Article history:
Received 10 September 2013
Received in revised form 8 October 2013
Accepted 8 October 2013
Available online 16 October 2013

Keywords:
Vitex negundo
Lagundi
MEKC
Chemical markers
Herbal products
Agnuside

ABSTRACT

Vitex negundo or lagundi is an important herbal medicine in Asia and various commercial products of this herb's leaves have been flooding the market. Reports on the chemical constituents of such products were limited and directed to only one selected chemical marker. Here, micellar electrokinetic chromatography using a fused silica capillary was used for analysis of the methanolic extract of Philippine lagundi herbal products. The separation solution, injection, and separation voltage was 50 mM SDS with 50 mM phosphoric acid at pH 2.5, 6 s at 25 mbar, and 20 kV at negative polarity, respectively. With UV detection at 200 nm, the LODs (S/N = 3) were in the sub-μg/mL range which were sufficient for detection without the need for on-line or off-line sample concentration. The repeatability values were all below 3.9% for migration time and peak signals. When the method was used to real samples, a flavonoid (isoorientin) and two iridoid glycosides (agnuside and negundoside) were identified as the target chemical markers within a 12 min electrophoretic run. The accuracy was from 95% to 129%. The results also suggested that agnuside was the major component in Philippine lagundi. The method was simple, effective, low cost, and can be used for authenticity evaluation of *lagundi* products.

Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.

1. Introduction

Vitex negundo Linn. (*Verbenaceae* family), a traditional herbal medicine, is an aromatic shrub mostly found around South and Southeast Asia. This shrub is called five-leaved chaste tree (English) and *huang jing* (Chinese) [http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?41831]. Locally known as *lagundi* in the Philippines, it is promoted by the country's Department of Health as an alternative remedy for respiratory problems such as colds, flu, pharyngitis, and asthma [1]. The *lagundi* leaves are commercially available as phytopharmaceutical or herbal products in the form of capsules, tablets, syrups, and teas. The products have been approved for the above health problems by the Bureau of Food and Drugs and an increasing number are flooding the local market.

The assessment of consistency, safety, and efficacy differs for pharmaceutical drug and herbal products. Pharmaceutical drug products contain one or more active pharmaceutical ingredients (API) of known amounts. The quality of drug products, including the levels of impurities, is strictly monitored in quality control/assurance laboratories. The herbal products on the other hand contain a cocktail of known and unknown active chemical compounds in unknown amounts. These compounds in herbal products are considered analogous to the APIs in pharmaceutical drug products. The quality and quantity of each compound vary depending on the source of the plant material, season of harvest, procedures in extraction and preparation, raw material adulterants or substitutes, among others.

Separation methods have been demonstrated for the quality control of herbal products [2–5]. The usual methods were high performance thin layer chromatography, high performance chromatography (HPLC), and gas chromatography. Several approaches such as the generation of a chemical fingerprint [6–10] or the use of a select group of chemical markers [11–15] were proposed. The former approach generates a unique and overall picture of the chemical constituents. The latter targets a single or group of chemical markers within the fingerprint. Although this approach had been criticized as being impractical and unrealistic (i.e., does not consider all constituents), it was considered attractive because it was convenient and fast. The choice of the chemical markers in this case was crucial. The chemical markers were ideally the unique components that contribute to the therapeutic effects of the herbal plant [16]; however other component classifications such as synergistic, characteristic, main, toxic, etc. were also used.

The *Vitex* genus contains a variety of potentially bioactive molecules, such as iridoids, flavonoids, diterpenoids, phytosteroids, etc. [17,18]. Any of these components are good candidates as chemical markers. Among the flavonoids, luteolin and isoorientin [19] were reportedly mostly abundant in the leaves. Also found abundant and already used as chemical markers in HPLC analyses were casticin [20] and vitexin

* Corresponding author at: Australian Centre for Research on Separation Science (ACROSS), School of Physical Sciences-Chemistry, University of Tasmania, Hobart, Tasmania, Australia. Tel.: +61 448 122 456; fax: +61 6266 2858.
E-mail address: jquirino@utas.edu.au (J.P. Quirino).

0026-265X/$ – see front matter. Crown Copyright © 2013 Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.microc.2013.10.006

[21], as well as the iridoid negundoside [22–25]. In recent years the popularity of capillary electrophoresis (CE) for the analysis of natural products has dramatically increased as a technique complementary to HPLC [3–5]. The two CE modes of capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are attractive phytochemical analysis tools due to minimum requirements for sample preparation, small sample size, low reagent consumption, high separation efficiency/selectivity, and fast analysis time [26–29]. These modes have been used to analyze chemical markers (e.g., a number of flavonoids which included luteolin, casticin, isoorientin, and vitexin [20,30–33]) for the quality control of different herbal plants and preparations. However, due to the complexity of herbal preparations there is no report yet on the use of CE or HPLC for the simultaneous determination of flavonoids, glycosides and other constituents in *Vitex negundo Linn*.

Here, we show the use of CE for the analysis of targeted chemical markers of Philippine *lagundi* herbal products. Reversed migration MEKC [34,35] was utilized for the separation of the chemical constituents including flavonoids luteolin, casticin, isoorientin, and vitexin and iridoid glycosides agnuside and negundoside. The identity of the selected chemical markers was studied using the UV spectrum of the peaks in the electrochromatograms. A simple solvent extraction with methanol was employed to real herbal products (capsules, tablet, and tea preparations). A longer run with the developed electrophoretic method was also studied for chemical profiling.

2. Materials and methods

2.1. Reagents and solutions

All reagents (phosphoric acid, sodium dodecyl sulfate, sodium hydroxide, and HPLC grade methanol) were purchased from Sigma Aldrich (St. Louise, MA). Solutions of 0.1 and 5 M NaOH and 0.2 M SDS were prepared by dissolving appropriate amounts of solids with purified water. The 0.5 M phosphoric acid at pH 2.5 was prepared by adjusting the pH of phosphoric acid with 5 M NaOH followed by dilution with water. All solutions were sonicated and filtered through a 0.45 μm filter from MicroScience (Co Durham, UK) prior to use. The separation solution consisted of 50 mM SDS and 50 mM phosphoric acid and prepared fresh each day.

The standard such as casticin was obtained from Indofine Chemical Co., Inc. (New Jersey, USA), luteolin, isoorientin, and vitexin from Sigma Aldrich (St. Louise, MA), and negundoside from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany). The in-house standard for agnuside was obtained as a gift from the Department of Chemistry, Ateneo de Manila University. The structures of these compounds are found in Fig. 1. The stock solutions (1 mg/mL) of the standards were all prepared in methanol. A suitability standard solution that contained 13.3–20.0 μg/mL casticin, luteolin, isoorientin, vitexin, and negundoside was prepared. This suitability standard solution was used with the real sample injections. The standard solution and samples were prepared by dilution with water.

2.2. Equipment

The CE instruments were a Beckman MDQ (Fullerton, CA) controlled using 32-Karat software and an Agilent 3D-CE controlled with Agilent Chemstation software. The UV detectors were set at 200 nm where all the chosen target chemical markers showed the best peak intensities. The fused silica capillaries with 50 μm i.d., 375 μm o.d., 60 cm total length (50 and 51.5 cm effective length for Beckman and Agilent instruments, respectively) were obtained from Polymicro Technologies (Phoenix, AZ). The capillary temperature was controlled at 20 °C during all experiments. New capillaries were conditioned with 0.1 M NaOH (30 min), water (10 min), and with separation solution (15 min) prior to use. The pH was measured using an Activon Model 210 pH meter (New South Wales, Australia). Water was purified with a Milli-Q system from Millipore (Bedford, MA).

2.3. General CE procedure

All electropherograms and electrophoretic data reported were generated using the Beckman system. The UV spectra were obtained using the Agilent system equipped with a diode array detector. Capillary conditioning was done after each run which involved rinsing with 0.1 M NaOH (1 min), water (2 min), and separation solution (5 min) by flushing at 950 mbar. Hydrodynamic injections of the standards mixture and real samples were done at 25 mbar for 6 s. The separation solution for reversed migration MEKC was 50 mM sodium dodecyl sulfate in low pH phosphate buffer (50 mM at pH 2.5). The low pH suppressed the EOF in fused silica capillaries. Separation voltage was at 20 kV (negative polarity or anode at the detector end).

2.4. Sample preparation

Commercially available *lagundi* herbal products were bought from reputable Philippine pharmacies and supermarkets. These products (as indicated in their labels) were suggested as remedy for cough and asthma. The supposed medicinal effect was claimed to come from the leaves. The brand U tablet forte, brand V tablet, brand W capsule forte were labeled L1, L2, L3, and tea bag brands X, Y, Z were labeled L4, L5, and L6, respectively. Forte means extra strength. For each tablet and capsule products, one tablet or capsule ($\approx$600 mg) of *lagundi* leaf was placed in a test tube. For the tea bag, approximately 600 mg of the bag contents was weighed and placed in a test tube. The products in the test tube were dissolved in 3.0 mL of methanol, sonicated for 1 h and centrifuged at 1200 rpm for 3 min. An aliquot of 1 ml was obtained and oven-dried for 30–60 min at 40 °C. The residue was reconstituted with 1 mL purified water, sonicated for 3 min, and then filtered through a 0.45 μm filter. The real sample solution was diluted with water such that the peak heights of the chemical markers were similar with the peak heights of the suitability standard solution injection.

3. Results and discussion

3.1. Reversed migration MEKC optimization for the selected chemical markers

Five chemicals (casticin, luteolin, isoorientin, vitexin, and negundoside) common in genus *Vitex* were selected as possible markers. These compounds are weak acids and have been analyzed as anions by CZE [20,30–33]. A different approach to achieve separation selectivity was then investigated using reversed migration MEKC where the compounds were analyzed as neutrals. Characteristic of this method was the use of micelles moving faster than and in the opposite direction of the suppressed EOF [34]. The EOF was suppressed by using a low pH buffer. At acidic pH, the five analytes were neutral. The analytes were carried to the detector by the micelles, thus the analytes with more affinity to the micelles were eluted faster than the analytes with less affinity. SDS was chosen as the micelle former because of its availability and proven usefulness for small molecules such as the chosen markers. Its selectivity is similar to a reversed phase HPLC.

It is known that SDS concentration in the separation solution affects the peak height and shape in MEKC. The effect of using 10 to 100 mM SDS in 50 mM phosphoric acid is shown in Fig. 2. In general, the peak heights improved while the retention times decreased with the increase in SDS concentration. When 10 mM SDS was used (see A), the peaks were broad and peak 5 eluted after 18 min. The peaks sharpened when the SDS concentration was > 10 mM (see B to E). Good separation of all markers with a reasonably short analysis time of 12 min was obtained with the 50 mM SDS condition (see C). The most hydrophobic casticin (peak 1) was detected first followed by luteolin (peak 2),

Download English Version:

https://daneshyari.com/en/article/7643445

Download Persian Version:

https://daneshyari.com/article/7643445

Daneshyari.com