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Simple and rapid procedures for the extraction of bioactive compounds from Guayule leaves

Fabrizio Piana[a], Marco Ciulu[a,*], Rosa Quirantes-Piné[b], Gavino Sanna[a], Antonio Segura-Carretero[b,c], Nadia Spano[a], Alberto Mariani[a]

[a] Università degli Studi di Sassari, Dipartimento di Chimica e Farmacia, Via Vienna 2, 07100 Sassari, Italy
[b] Research and Development Functional Food Center (CIDAF), Health Science Technological Park, Avenida del conocimiento 37, Edificio Bioregión, 18016 Granada, Spain
[c] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Avenida Fuentenueva S/N, 18071 Granada, Spain

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ABSTRACT

Solid liquid extraction (SLE) and ultrasound-assisted extraction (UAE) using food grade solvents were applied to Guayule leaves in order to obtain phenolic compound-rich extracts. The influence of solvent composition and time were evaluated for both techniques reaching the highest phenolic content with UAE using water and 25% aqueous ethanol (27.0 ± 0.2 and 22 ± 2 mg gallic acid equivalents/g dried leaves, respectively) and with SLE using water and 50% aqueous ethanol (16 ± 1 and 17 ± 1 mg/g, respectively). Antioxidant and antiradical activities of the selected extracts were evaluated by means of spectrophotometric procedures. In addition HPLC-ESI-QTOF-MS was used to characterize the bioactive compounds in the richest extracts. A variety of phenolic compounds, mainly phenolic acids and flavonoids, were identified for the first time in Guayule leaves. This study indicates that conventional extraction techniques, such as SLE and UAE, represent a powerful tool for the fast isolation of antioxidant compounds from Guayule.

1. Introduction

Guayule (*Parthenium argentatum* Gray) is a shrub belonging to the Asteraceae family, native to the Chihuahua desert (Mexico) and the southwest regions of the United States. The reason of the strong industrial and scientific interest towards this plant lies in the possibility to extract a natural rubber which can be used as hypoallergenic latex. Nowadays, guayule rubber is employed for the fabrication of medical and hygiene products (gloves, condoms, wetsuits *etc*.) and it also showed a great potential as raw material for tire production (Rasutis et al., 2015). Notwithstanding the environmental and economic sustainability of Guayule rubber, industries and researchers are always looking for new strategies to reduce manufacturing costs by the valorization of by-products. Since the woody parts of the shrub represent the source of the natural rubber, the extraction generally implies a preliminary defoliation of the plant. The general aim of this work was to verify the possibility to exploit Guayule leaves, which constitute a waste by-product in the industrial production of natural rubber, as a source of phenolic compounds.

Phenolic compounds are renowned for their health-promoting and physiological properties. They are secondary metabolites synthesized by plants that play a key role in many aspects related to the growth and survival of the plants themselves such as the reproduction mechanisms, the defense against predators and the development of the morphological characteristics (Balasundram et al., 2006 and refs therein). Among the functional properties for which these compounds are so important we can include the anti-microbial, the antioxidant and the anti-inflammatory activities. In addition, the inclusion of polyphenols in the quotidian diet has proved to have a positive effect in the prevention of cardiovascular and neurodegenerative diseases (Vauzour et al., 2010 and refs therein). Scientific literature offers only one example regarding the characterization of the phenolic fraction of Guayule leaves. In the work by Mears (1980), the extraction of phenolic compounds from Guayule dried leaves is performed by means of a 24 h maceration using a methanol/water (85/15, v/v) mixture. The solution is then filtered, dried and separated in a chloroform soluble fraction and a methanol-water-soluble fraction. Identification of the analytes in the two fractions is achieved by two-dimensional paper chromatography. Nevertheless the analytical technique chosen for the identification of the phenolic compounds doesn't allow a certain attribution of the analytes, this study can be considered as pioneer since it constitutes the first and only example of extraction and characterization of polyphenols from Guayule leaves.

Solid-liquid extraction (SLE), more commonly known as

* Corresponding author.
E-mail address: mciulu@uniss.it (M. Ciulu).

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maceration, represents one of the most traditional ways to isolate bioactive compounds from vegetal tissues. The sample is generally ground to a fine powder in order to facilitate the contact with the selected solvent while shaking is necessary to enhance the diffusion of the analytes from the sample to the extraction medium. In addition, shaking helps removing the layer of concentrated solution from the sample surface improving the extraction yield (Azmir et al., 2013).

Ultrasound-assisted extraction (UAE) is another useful way to obtain extracts rich in bioactive compounds from plants. In this case, the diffusion of the analytes from the vegetal cells to the solvent is promoted by the application of a radiation with a frequency included between 20 kHz and 100 MHz which is responsible for the cavitation phenomena and promotes the rinsing of the cell walls (Mason et al., 1996; Azmir et al., 2013).

Both techniques are simple, fast and not expensive and are valuable for the isolation of phenolic compounds from plant tissue. In addition, they can be easily applied on industrial-scale. Given the scarceness of information about the phenolic fraction of Guayule leaves, the specific purposes of this work were: 1) the evaluation of the potential of simple, rapid and not expensive techniques for the extraction of phenolic compound-rich extracts from Guayule leaves; 2) the spectrophotometrical characterization of the richest extracts by means of tests aimed to the quantification of flavonoids and the evaluation of functional properties such as the antioxidant and the antiradical activity; 3) the preliminary characterization of the bioactive compounds of the selected extracts by HPLC-ESI-QTOF-MS. For this study only food grade solvents (*i.e.* water and ethanol) were used.

2. Materials and methods

2.1. Chemicals

HPLC–MS grade acetonitrile and acetic acid were purchased from Fisher (Thermo Fisher Scientific, Leicestershire, UK). Ethanol, gallic acid, quercetin, 2,2-diphenyl-1-picrylhydrazyl, ferrous sulfate and Folin–Ciocalteu reagent were from Sigma–Aldrich (Steinheim, Germany). Ascorbic acid was obtained from Lancaster (Eastgate White Lund, Morecambe, England). Sodium carbonate was purchased from Carlo Erba (Milan, Italy). Aluminium chloride was provided by Fluka (Milan, Italy). Ultrapure water (resistance of 18.2 MΩ) was obtained by a Milli-Q system (Bedford, MA, USA).

2.2. Plant material

Guayule plants were harvested in 2016 in the desert of Chihuahua (Mexico). Leaves were preventively separated from the other parts of the shrub and ground to a fine powder by mortar. The plant material was homogenized and stored at −18 °C before analysis.

2.3. Extraction of phenolic compounds from Guayule leaves

Some selected conditions were tested for solid-liquid extraction (SLE) and ultrasounds-assisted extraction (UAE). In both cases, the effect of solvent composition and time was evaluated. Only generally recognized as safe (GRAS) solvents (water and ethanol) were used.

2.3.1. Testing of SLE conditions

Each sample (2.5 g) of leaf powder was introduced in a beaker and 25 mL of the extraction mixture were subsequently added. Ethanol and water were chosen as components of the extraction medium. Five different ethanol percentages (0%; 25%; 50%; 75%; 100%; v/v) were tested in order to select the best solvent composition for the extraction of phenolic compounds. All the extractions were performed in triplicate, at room temperature and by covering the mixture with an aluminum foil in order to protect the analytes from the light. An extraction time of 1 h was chosen.

After selecting the best ethanol percentages (0% and 50%) the effect of time was studied by performing the extractions also in 3 and 6 h.

After each extraction, the suspensions were centrifuged (ST16R Centrifuge, Thermo Fisher Scientific, Leicestershire, UK) at 10000 rpm for 15 min, the supernatants were filtered (0.45 μm) and evaporated under vacuum.

2.3.2. Testing of UAE conditions

Each sample (2.5 g) of leaf powder was introduced in a beaker and 25 mL of the extraction mixture were subsequently added. A frequency of 35 kHz was adopted for the tests. Ethanol and water were chosen as components of the extraction medium. Five different ethanol percentages (0%; 25%; 50%; 75%; 100%; v/v) were tested in order to select the best solvent composition for the extraction of phenolic compounds. All the extractions were performed in triplicate, at room temperature and by covering the mixture with an aluminum foil in order to protect the analytes from the light. An extraction time of 30 min was chosen.

After selecting the best ethanol percentages (0% and 25%) the effect of time was studied by performing the extractions also in 15, 45 and 60 min. After each extraction, the suspensions were centrifuged (ST16R Centrifuge, Thermo Fisher Scientific, Leicestershire, UK) at 10000 rpm for 15 min, the supernatants were filtered (0.45 μm) and evaporated under vacuum.

2.4. Determination of total phenolic content

Total phenolic content of all the extracts was quantified by the Folin-Ciocalteu's assay as described by Rodríguez-Pérez et al. (2015), in order to select the best extraction conditions. Each dried extract was dissolved in an appropriated volume of its correspondent extraction solvent to obtain solutions at a concentration of 1 mg/mL. 600 μL of water and 10 μL of extract or standard solution were mixed with 50 μL of Folin–Ciocalteu reagent. After 10 min, 150 μL of 2% (w/v) Na_2CO_3 was added and the volume was made up to 1.0 mL with water. After 2 h of incubation at room temperature in darkness, 200 μL of the mixture was transferred into a well of a microplate. The absorbance was measured at 760 nm using a Synergy Mx Monochromator Based Multi-Mode Micro plate reader (BioTek Instruments, Winooski, VT, USA) and compared to a gallic acid calibration curve (5–150 μg/mL) elaborated in the same manner. The total phenolic content was calculated as mean ± SD (n = 6) and expressed as mg of gallic acid per g of dry leaves.

2.5. Spectrophotometric characterization of the extracts

The extracts richest in phenolic compounds obtained by the procedures previously described were characterized in order to determine the total content of flavonoids along with the antioxidant and antiradical activity. All the analyses were performed in triplicate.

2.5.1. Total content of flavonoids

Total content of flavonoids of the richest extracts were spectrophotometrically determined by means of the procedures described by Slinkard and Singleton (1977). Derivatization of flavonoids was performed by mixing 0.4 mL of extract (1 mg/mL) to 0.2 mL of an aqueous solution of $AlCl_3$ (5%, m/v) and diluted with ethanol up to 10.00 mL. Calibration was performed with quercetin and the absorbance of the extracts was determined after 30 min from the derivatization. Results were expressed as mg of quercetin per g of dried leaves.

2.5.2. Antioxidant and antiradical activity

Total antioxidant capacity of the richest extracts was determined by evaluating the ferric reducing/antioxidant power (FRAP) as described by Benzie and Strain (1996). FRAP assay measures the change in absorbance at 593 nm due to the formation of a blue colored Fe (II)-tripyridyltriazine compound from colorless oxidized Fe(III). Calibration