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Ultrasound-assisted extraction of polyphenols from native plants in the Mexican desert

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ABSTRACT

Several plants that are rich in polyphenolic compounds and exhibit biological properties are grown in the desert region of Mexico under extreme climate conditions. These compounds have been recovered by classic methodologies in these plants using organic solvents. However, little information is available regarding the use of alternative extraction technologies, such as ultrasound. In this paper, ultrasound-assisted extraction (UAE) parameters, such as the liquid:solid ratio, solvent concentration and extraction time, were studied using response surface methodology (RSM) for the extraction of polyphenols from desert plants including *Jatropha dioica, Flourensia cernua, Turnera diffusa* and *Eucalyptus camaldulensis*. Key process variables (i.e., liquid:solid ratio and ethanol concentration) exert the greatest influence on the extraction of all of the phenolic compounds (TPC) in the studied plants. The best conditions for the extraction of TPC involved an extraction time of 40 min, an ethanol concentration of 35% and a liquid:solid ratio ranging from 8 to 12 ml g^{-1} depending on the plant. The highest antioxidant activity was obtained in the *E. camaldulensis* extracts. The results indicated the ability of UAE to obtain polyphenolic antioxidant preparations from desert plants.

1. Introduction

Mexico is one of the five richest countries in a large variety of plants in the world. Mexico contains 25,000 registered species and approximately 30,000 species that have not yet been described [1,2]. In addition, more than 3500 native medicinal plant species have been identified and classified [3]. In this context, the desert region in northern Mexico is important due to the great variety of plants that have developed the ability to grow under extreme climate conditions with a variety of chemical compounds that are used as defence mechanisms [4]. Desert scrub covers three semiarid areas with the greatest biodiversity in the world [5].

Several studies have been performed using plant species from the Mexican desert to study the potential biological activities of these plants as inhibitors with pathogenic bacterial [6], postharvest fungal [7–12] and antioxidants properties [13,14].

These compounds have been extracted using conventional techniques, such as maceration, infusion or reflux, that exhibit disadvantages, such as high solvent requirements, long extraction times and risks of degradation of the thermo-labile constituents [15,16]. In addition, the solvents commonly employed include methanol, hexane, and acetone, and in recent years, unconventional solvents, such as lanolin and cocoa butter, as well as organic solvents, such as water and ethanol [4,17], have been used, showing the same effects against bacterial and fungal pathogens.

Therefore, within the context of being environmentally friendly and sustainable, alternative technologies and solvents are being studied to perform extractions at same or lower cost while increasing the extraction quality [18,19]. Various novel extraction techniques have been investigated that resolve some of the shortcomings of the conventional techniques. Ultrasound-assisted extraction (UAE) is an example of an alternative extraction technique [20]. UAE offers many advantages, such as selectivity, high efficiency and productivity, enhanced quality, low energy, reduced extraction time and solvent consumption, reduced chemical and physical hazards, environmentally friendly, inexpensive and high level of automation, compared to conventional extraction techniques [21–24].

Research on antioxidants has received increasing interest because natural antioxidants are gaining importance due to their benefits for human health compared to synthetic antioxidants,

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which can produce serious side effects [25]. Polyphenols are the major plant compounds associated with healthy properties due to their antioxidant activity and free radical scavenging ability [26,27], and several studies have demonstrated their antimicrobial activity, which make them a good alternative to chemical preservatives [28]. For example, desert Mexican plants are rich in polyphenols (i.e., tannin) [4,17].

To the best of our best knowledge, there are no reports on the use of UAE to extract phenolic compounds from semiarid Mexican plants, and only poor information about the antioxidants is known. Therefore, the aims of the current study were to study the extraction efficiency of UAE of polyphenolic compounds from the *Jatropha dioica, Flourensia cernua, Turnera diffusa* and *Eucalyptus camaldulensis* plants and to evaluate the antioxidant potential of the obtained extracts. In UAE, several extraction variables, such as ultrasonic power, temperature, sonication time, and solvent concentration and ratio, could be explored. In the current research, we chose to study the extraction time, liquid–solid ratio and ethanol concentration to evaluate the feasibility of the method.

2. Materials and methods

2.1. Reagents and vegetable materials

The *J. dioica* stem and root, *F. cernua* stem and leaves, *E. camaldulensis* leaves and *T. diffusa* stem and leaves were collected from January to June 2011 in places near Saltillo City, Coahuila, Mexico. The plants were dried (48 h at 60 °C, Oven LABNET International, Inc.), pulverized into powder and sieved (0.6–0.8 mm particle size). The fine and dried powder was stored at room temperature in hermetically sealed bags under darkness prior to use. The acetonitrile (ACN), acid acetic, methanol and ethanol solvents were of analytical grade. The standards used (i.e., pyrogallol (PG), gallic acid (GA), resorcinol (RS), chlorogenic acid (CHA), methyl gallate (MG), coumaric acid (CUA), catechin (CAT), 2-hydroxycinnamic acid (HA), ellagic acid (EA), quercetin (QE), cinnamic acid (CA) and 1,1-diphenyl-2-picrylhydrazil (DPPH) free radical, linoleic acid, Folin–Ciocalteu (FC) reagent) were purchased from Sigma–Aldrich.

2.2. Ultrasound-assisted extraction (UAE)

The ultrasound-assisted extraction was performed in an ultrasonic bath device (Model 2510, BRANSON). The bath consisted of a rectangular container (34.29 cm × 10.16 cm × 30.5 cm) with 40 kHz transducers annealed to the bottom. The samples were processed at room temperature. The ultrasonic wave provided a slight increase in the temperature (i.e., between 40 and 50 °C).

The powder of the dried plants (4 mg) was placed in a capped tube (100 mL) and mixed with an appropriate amount of the extraction solution (according to experimental design). Next, the tube with the suspension was immersed in water in the ultrasonic device and irradiated for the pre-set extraction time. Then, the obtained extracts were filtered (Whatman No. 41 paper) and centrifuged at 3500 rpm for 10 min. Next, the extracts were dehydrated at 60 °C for 48 h and stored under refrigeration prior to the TPC analysis. For all of the determinations, the extracts were resuspended in water (1 mg of dried extract ml^{-1}).

2.3. Optimization of process parameters

The extraction of the total phenolic content of the plants by ultrasound was performed by employing various extraction conditions. In this study, a complete factorial (3^3) with three levels and three factors was applied to determine the best combination of extraction variables. The factors selected included extraction time, liquid–solid ratio and ethanol concentration (Table 1). All of the treatments were performed in triplicate.

Response surface methodology (RSM) is a collection of statistically based experimental designs that have been established as a convenient method for optimizing several processes [29]. Therefore, RSM was used to analyse the results along with the Statistica software (Statsoft version 7.0). The calculations were performed at the 95% confidence level. The optimal extraction conditions were estimated with the regression analysis performed on the data for the dependent variable obtained and were fitted to an empiric polynomial model, as shown in the following general equation:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3$$

where y is the predicted response, b_0 is the interception, b_1, b_2 and b_3 are the linear coefficients for the ethanol concentration (x_1), ultrasonic time (x_2) and liquid–solid ratio (x_3), respectively, b_{11}, b_{22} and b_{33} are the squared coefficients for the ethanol concentration, ultrasonic time and liquid–solid ratio, respectively, and b_{12}, b_{13} and b_{23} are the interaction coefficients for the ethanol concentration, ultrasonic time and liquid–solid ratio, respectively.

2.4. Yield of total phenolic content (TPC)

The total phenolic compounds were analysed using the traditional Folin–Ciocalteu method with some modifications [8,30]. First, the traditional method was adapted for small volumes with microplate assays to provide exhaustive standardization for the modified technique (data not shown). Then, 20 μL of each extract (1 mg ml^{-1}) was mixed in a well with 20 μL of the Folin and Ciocalteu's reagent. After 5 min, 20 μL of Na_2CO_3 (0.01 M) was added to each sample and allowed to stand for 5 min. Next, the solution was diluted with 125 μL of distilled water, and the absorbance was read at 790 nm using a spectrophotometer microplate reader

Table 1
Complete factorial design employed for the extraction of TPC.

Run	Code variables			Decoded variables		
	X_1	X_2	X_3	Ethanol concentration (%)	Time (min)	Liquid:solid ratio (ml/g)
1	−1	−1	−1	0	20	4
2	−1	−1	0	0	20	8
3	−1	−1	1	0	20	12
4	−1	0	−1	0	40	4
5	−1	0	0	0	40	8
6	−1	0	1	0	40	12
7	−1	1	−1	0	60	4
8	−1	1	0	0	60	8
9	−1	1	1	0	60	12
10	0	−1	−1	35	20	4
11	0	−1	0	35	20	8
12	0	−1	1	35	20	12
13	0	0	−1	35	40	4
14	0	0	0	35	40	8
15	0	0	1	35	40	12
16	0	1	−1	35	60	4
17	0	1	0	35	60	8
18	0	1	1	35	60	12
19	1	−1	−1	70	20	4
20	1	−1	0	70	20	8
21	1	−1	1	70	20	12
22	1	0	−1	70	40	4
23	1	0	0	70	40	8
24	1	0	1	70	40	12
25	1	1	−1	70	60	4
26	1	1	0	70	60	8
27	1	1	1	70	60	12