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Green analytical chemistry metrics: Towards a sustainable phenolics extraction from medicinal plants

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ABSTRACT

Green metrics quantify the sustainability of chemical processes. Since their introduction many years ago, this topic has been an active area of research and discussion. However, the application of these tools as additional criteria for method selection is still unexploited. In the present work, a comparative study was carried out in order to assess the greenness of the most recent approaches for phenolics extraction from medicinal plants using the Green metrics. Considering the obtained results, a green and efficient Natural Deep Eutectic Solvent (NADES) mediated-ultrasound extraction for phenolic compounds was developed and applied to an Argentinian autochthonous plant (*Larrea cuneifolia*) commonly used in folk medicine. To this purpose, experimental conditions were chemometrically optimized to maximize efficiency and contribute to greening the approach. The results reveal that the proposed approach showed total penalty points similar to those obtained with water. Finally, the performance of NADES as extracting agent was compared with traditional solvents showing outstanding extractability for both polar and weak polar phenolics.

1. Introduction

As old as humankind, plants have been used in folk medicine since they are a rich source of natural bioactive compounds [1, 2]. In Argentina, autochthonous plants belonging to the genus *Larrea* (Zygophyllaceae) are one of the most remarkable used in folk medicine. Among *Larrea* species, *L. cuneifolia* has been traditionally used as anti-inflammatory, antirheumatic, dysphoretic, amenagogic and antimicrobial agents. However, there is lack of information concerning their chemical composition [3, 4]. Bioactive compounds from plants are mainly secondary metabolites, being the phenolic compounds one of the most relevant groups. Interestingly, they have been explored for their biological activities such as antimicrobial, antioxidant and anti-inflammatory [5, 6]. Extraction of plant phenolic compounds is traditionally performed by conventional techniques such as maceration, soxhlet, decoction and infusion [7–10], most of them are recommended by Pharmacopoeias or Official Methods of Analysis of the AOAC [11]. However, they present some drawbacks such as long extraction periods, high solvent and energy consumption that makes them harmful from an environmental perspective [12]. Non-conventional techniques, including microwave-assisted (MW) and ultrasound-assisted extraction (UAE), have gained interest since they reduce the use of toxic organic solvents, improving sample throughput and efficiency [13–15].

The selection of a suitable solvent is crucial to improve the extraction efficiency. Water and organic solvents, such as ethanol, methanol, and isopropanol, are the most commonly used [16]. Nevertheless, water is only effective as extraction solvent for polar compounds. On the other hand, organic solvents are efficient for extract polar and weak polar compounds. However their toxicity, environmental hazardous, high cost and low biodegradability extremely limit their applications. Thus, the search for sustainable and safe alternatives for replacing toxic organic solvents without compromising efficiency is of utmost important [17].

Natural Deep Eutectic Solvents (NADES) has emerged in the last decade as promising green media. These novel green solvents are constituted of metabolites that are naturally present in all types of cells and organisms. The common components are sugars (glucose, sucrose, fructose, etc.); organic acids (lactic, malic, citric acids, etc.); urea and choline chloride. From an environmental and economic perspective NADES offer many remarkable advantages including biodegradability, low toxicity, solute stabilization, sustainability and low cost [18].

Considering the aforementioned, a quantitative tool to know how green is a “green analytical methodology” is essential. Since 2002, different green metrics have been proposed in order to evaluate the

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greenness of an analytical methodology. Recently in 2016, De la Guardia and co-workers [19] proposed the "Green Certificate", that is based on the application of weighted penalty points and the use of a colour code. Noteworthy, this metric consider the scale of method applications (micro-, meso- and macroscale), solving the problems of its predecessors. Parameters such as reagent toxicity and volume, energy consumption and the amount of wastes generated in the extraction step are included in this evaluation. Even though the Green metrics are a simple tool of ease application, only few reports have contemplated them to evaluate the analytical procedure sustainability.

In the present work, a comparative study was carried out in order to evaluate the greenness of the most recent procedures for phenolic compounds extraction from medicinal plants using the Green Certificate. Considering the obtained results, a green and efficient NADES based-ultrasound mediated extraction of phenolic compounds from *Larrea cuneifolia* was developed. To this purpose, experimental conditions were chemometrically optimized for maximized efficiency. Finally, the performance of NADES as extracting agent was compared with traditional solvents.

2. Materials and methods

2.1. Plant material

Larrea cuneifolia was cultivated at a greenhouse under natural radiation and was identified by means of morphological, anatomical, and histochemical analyses. Leaves were harvested during flowering period and immediately frozen in liquid nitrogen. Then they were lyophilized in darkness. Before the extraction, lyophilized material was grounded up to a fine powder with liquid nitrogen.

2.2. Chemicals, standards solutions and equipment

Analytical standards, apigenin 95% (Api), cinnamic acid ≥99% (Cin), (±)catechin hydrate (Cat), naringenin ≥95% (Nar), caffeic acid ≥99% (Caf), nordihydroguaiaretic acid ≥97% (NDGA) and rosmarinic acid ≥99% (Ros) were purchased from Sigma Aldrich (St. Louis, MO,USA). Quercetin dihydrate ≥97% (Quer) were obtained from Alfa Aesar (Haverhill, MA, USA); tyrosol > 99,5% (Tyr), luteolin ≥98% (Lut), rutin hydrate ≥94% (Rut) from Fluka Analytical (St. Louis, MO,USA); and trans-ferulic acid ≥99% (Fer) from SAFC (St. Louis, MO,USA). Compounds for NADES preparation including glucose anhydrous (≥99%) and L(+)lactic acid (85–90%) were purchased from Biopack (Bs. As., Argentina). Ultrapure water was obtained from a Milli-Q system (Millipore, Billerica, MA, USA). Methanol (MeOH) and acetonitrile (ACN) of chromatographic grade was purchased from J. T. Baker (USA). Formic acid (85%) (FA) was obtained from Sintorgan (Bs. As., Argentina). Stock solutions were prepared by dissolving each phenolic standard at concentration of 1000 μg mL^{-1} in NADES, methanol (MeOH) or water (H_2O). Lut, Quer and NDGA were prepared only in NADES and MeOH. Standard working solutions at concentration of 50, 25, 15, 5 μg mL^{-1} were obtained from stock solutions. All these solutions were stored in dark-glass bottles at 4 °C.

Magnetic stirrer with temperature control Fisatom (model 752A, Brasil) was used in the preparation of NADES. For extraction of phenolic compounds from *L. cuneifolia* a centrifuge (Eppendorf 5804-R) and ultrasonic bath (Cleanson-Buenos Aires; 200 W output power, 20 kHz frequency) were used.

2.3. NADES preparation

The NADES was prepared using a method previously described by Dai and co-workers [20]. The components mixture (lactic acid and dextrose; 5:1) with 15% of H_2O (v/v), named as LGH, was placed in a 20 mL amber glass vial. After, the mixture was heated in a magnetic stirrer with temperature control at 40 °C for 60 min.

2.4. Larrea cuneifolia NADES extraction

The extraction of phenolic compounds from *L. cuneifolia* with LGH, was performed mediated by Ultrasound Assisted Extraction (UAE) and optimized using multivariate analysis. The parameters evaluated were ultrasound time (15, 38, 60 min), sample material/solvent ratio (15, 45 and 75 mg mL^{-1}) and water dilution of LGH (0%, 38% and 75%). As described in Section 2.3, LGH was prepared with 15% of water; the proposed dilutions in the extraction optimization were performed afterwards. The extraction temperature was set at 40 °C to avoid degradation. The chromatographic area of selected phenolic compounds was used to evaluate the extraction performance. As a result of the optimization, lyophilized material and LGH were placed in a 15 mL centrifuge tube (75 mg mL^{-1}) and homogenized by a vortex during 15 s. The suspensions were processed by ultrasound (200 W output power, 20 kHz frequency) during 42 min at 40 °C (± 2 °C). Then, the system was centrifuged at 10000 rpm for 30 min and the supernatant were filtered (0.45 μm) before analyzed by HPLC-DAD. Each extraction was performed in triplicate.

2.5. Experimental design and statistical analysis

The UAE method was evaluated using Box-Behnken Design. In order to evaluate and optimize the extraction parameters, Response Surface Methodology was applied. The statistical analysis was performed using the RSM software Design-Expert, v.7.1 (Stat Ease, Minneapolis, USA). The results were tested statistically using analysis of variance (ANOVA) at the significance level of $p = 0.05$. The adequacy of the model was evaluated by the coefficient of determination (R^2) and the model p value. Mathematical models were established to describe the influences of the single process parameters and/or the interactions of multiple parameters on each response investigated. Response surface plots were generated with the same software, and were drawn using the function of two factors, and keeping the other factor constant. Statistical analysis was done using software Statgraphics Centurion XV v15.2.06 and GraphPad Prism v5.01. All data were reported as the mean ± SD for three replicates.

2.6. HPLC-DAD analysis

Phenolic compounds were determined using a HPLC-DAD system (Dionex Softron GmbH, Thermo Fisher Scientific Inc., Germering, Germany). The HPLC instrument was a Dionex Ultimate 3000 consisting of vacuum degasser unit, autosampler, quaternary pump and chromatographic oven. The detector was a Dionex DAD-3000 (RS) model. Chromeleon 7.1 software was used to control all the acquisition parameters of the HPLC-DAD system and also to process the obtained data. HPLC separations were carried out in a Zorbax SB-Aq column (4.6 mm × 150 mm, 5 μm) Agilent Technologies. Ultrapure water with 0.1% FA (A) and ACN (B) were used as mobile phase. Phenolic compounds were separated using the following gradient: 0–3 min, 5% B; 3–11 min, 15% B; 11–15 min, 32% B; 15–15.5 min, 40% B; 15.5–16 min, 50% B: 16–16.5 min 50% B; 16.5–17 min 30% B; 17–17.5 min 15% B; 17.5–18.5 min 5% B; 18.5–20 min 5% B. The mobile phase flow was 1.2 mL min^{-1}. The column temperature was held at 30 °C and the injection volume was 5 μL. The identification and quantification of phenolic compounds for the studied solvents were based on the comparison of the retention times (tR) and absorbance values of detected peaks in solvents with those obtained by injection of pure standards of each analyte. Chromatograms were recorded at 280 (Tyr, Cat, Cin, Nar, NDGA), 320 (Caf, Fer, Ros, Api) and 370 nm (Rut, Quer, Lut).

2.7. Green metrics

Penalty Points (PPs) were calculated according to the "Green

Certificate" proposed by de la Guardia and co-workers [19]. The parameters evaluated were reagent (type and volume), energy consumption and waste generation.

Penalty points for reagent (PP_R) and waste volume (PP_W) were calculated according to the following Eqs. (1) and (2):

$$PP_R = (0.61 \pm 0.05)\, V^{(0.31\pm0.02)} \quad (1)$$

$$PP_w = (0.50 \pm 0.08)\, W^{(0.4\pm0.02)} \quad (2)$$

where, V: reagent volume and W: waste volume.

Penalty points (PP) for energy consumption were assigned according to the values proposed by Raynie and Driver [21] regarding the power involved in sample analysis. Less or equal than 0.1 kWh per sample involves 1 PP, between 0.1 and 1.5 kWh per sample concerns 2 PP, and > 1.5 kWh per sample 3 PP.

3. Results and discussion

3.1. Green metrics

Extraction is a key step for the isolation of biocompounds from plants matrices. A comparative study was carried out with the aim to evaluate the greenness of the most recent approaches for phenolic compounds extraction from medicinal plants (Table 1). Penalty points were calculated following the Green Certificate, as described in Section 2.7.

Analysing the results, conventional techniques PPs range from 9.53 to 25.11 whereas nonconventional present a significant PPs reduction since they range from 3.5 to 13.46. As can be seen, the higher PPs of conventional ones are due principally to the large volumes and the type of organic solvents they applied. Also, these techniques are more energy consuming and generate higher amounts of waste. Another critical aspect to keep in mind is that conventional techniques are time consuming, representing an important disadvantage to practical purposes. On the other hand, nonconventional techniques such as microwave and ultrasound are notably better from a green perspective.

With the need to reduce reagent PPs, water appears as a sustainable alternative for phenolic compounds extraction. However, a rational way to optimize extraction techniques is not only to minimize the environmental impact but also maximize efficiency. Taking this into account, several reports compared the extraction yields for phenolics demonstrating that water is less efficient than organic solvents since it is only effective for polar and hydrophilic bioactive compounds [10, 22]. Lately, a great number of alternative solvents have gained

Table 1
Green Certificate for phenolic compounds extraction methods from medicinal plants.

	Extraction							Green Certificate[b]	Reference
	Extraction technique	Reagent			Energy ($kW\,h^{-1}$)	Waste (PP_w)	Total PPs		
		Reagent amount (mL) PP_R	Hazard- (PP_{RH})	Subtotal PP[a]					
Conventional techniques	Heat-reflux	1.005 (5 mL de MeOH)	6	6.028	2	1.5	9.53	90.47	[25]
	Maceration (with agitation)	1.42 (15 mL MeOH)	6	8.474	2	0.653	11.13	88.87	[21]
	Decoction	– (200 mL H_20)	–	–	8	4.972	12.97	87.03	[8]
	Maceration	1.427 (15.5 mL MeOH)	6	8.560	1	1.500	13.68	86.32	[9]
		0.654 (1.25 mL acetic acid)	4	2.617					
	Maceration (with agitation)	1.634 (24 mL MeOH)	6	9.803	4	1.137	14.94	85.06	[27]
	Maceration (with agitation)	2.082 (52.5 EtOH)	4	8.329	–	2.855	15.19	84.19	[4]
	Maceration (24horas)	2.543 (100 mL MeOH)	6	15.257	2	2.855	20.11	79.89	[6]
	Maceration	2.543 (100 mL MeOH)	6	15.257	4	2.855	22.11	77.89	[10]
	Soxhlet	2.543 (100 mL MeOH)	6	15.257	7	2.855	25.11	74.89	[10]
Non-conventional techniques	Ultrasound	– (100 mL H_2O)	–	–	2	1.5	3.50	96.5	[5]
	Ultrasound	– (10 mL H_2O)	–	–	3	0.788	3.79	96.21	[14]
	Ultrasound	– (1 mL NADES)	–	–	3	0.34	7.97	92.03	[23]
		0.94 (4 mL ACN)	6	5.62					
	Microwave	1.005 (5 mL de MeOH)	6	6.028	1	1.5	8.53	91.47	[25]
	Ultrasound	1.473 (17.200 mL EtOH)	4	5.894	3	1.5	10.39	89.61	[12]
	Ultrasound	– (1 mL NADES)	–	–	3	1.20	13.46	86.54	[24]
		1.54 (20 mL MeOH)	6	9.26					
	Ultrasound	– **(5 mL LGH)**[c]	–	–	**3**	**1.01**	**4.01**	**95.99**	**Present study**

[a] Subtotal PP = PP_R PP_{RH}.
[b] Green certificate = 100 − Total PPs.
[c] Present approach.

Table 2
Experimental data of the responses tested in the extraction of *Larrea cuneifolia* phenolic compounds by Box-Behnken Design (BB).

Run	Factors			Responses		
	Plant/solvent ratio (mg mL^{-1})	NADES dilution	Extraction time	Caf	Fer	Lut
1	15	38	15	0.074	0.498	28.219
2	75	75	38	0.464	2.341	75.288
3	45	38	38	0.320	15.081	73.544
4	75	38	60	0.431	28.461	14.667
5	45	38	38	0.356	16.356	92.083
6	75	0	38	0.362	17.965	140.932
7	45	38	38	0.395	17.698	10.555
8	15	38	60	0.087	0.608	3.666
9	45	0	15	0.248	11.305	104.629
10	75	38	15	0.453	25.909	155.271
11	45	75	60	0.315	15.347	5.817
12	45	38	38	0.317	16.016	80.353
13	45	38	38	0.289	15.107	79.207
14	15	0	38	0.081	0.383	34.948
15	45	38	38	0.346	1.618	103.566
16	15	75	38	0.044	0.434	13.396
17	45	75	15	0.2578	13.159	36.762
18	45	0	60	0.2121	10.363	86.472

attention due to their sustainability and efficiency. Among them, Natural Deep Eutectic Solvents have demonstrated satisfactory features. However, most of the reports that include these green solvents for extraction, also use organic solvents in subsequent steps representing a misalignment from Green Chemistry concept [23, 24].

3.2. *Larrea cuneifolia*-NADES extraction

Considering the comparative study developed in the previous section, a NADES based-ultrasound mediated extraction was selected in order to reduce the PPs, achieving high phenolic extraction yields.

A considerable number of variables affect the efficiency of the extraction, thus the optimization was carried out through a multivariate approach as described in Section 2.4. For the optimization of extraction conditions, natural sample of *L. cuneifolia* was used. Box-Behnken experimental design (CCD) with Response Surface Methodology (RSM) was used to determine the optimal experimental conditions (plant-solvent ratio, NADES dilution and ultrasound time) for phenolic compounds extraction from *L. cuneifolia*. The design consisted of eighteen experiments with six replicates of center points as can been seen in Table 2.The response variables were the chromatographic area of luteolin, ferulic and caffeic. These phenolic compounds were selected considering their chemical nature and polarity as well as their occurrence in *L. cuneifolia* extract.

The models that better explained the behavior of responses were quadratic model for ferulic and caffeic acids and linear model for luteolin (Table 3). The resulting R^2 indicates that the experimental data were in satisfactory agreement with predicted responses for each model. F-values for the lack-of-fit of linear model were not significant, indicating that this model accurately represents the experimental data.

Table 3
ANOVA statistics of the selected models.

Response	Model	p-Value*	p-Value*	R^2 adj.
		Model	Lack of fit	
Ferulic acid	Quadratic	< 0.0001	0.2784	0.9857
Caffeic acid	Quadratic	< 0.0001	0.5183	0.9227
Luteolin	Linear	< 0.0001	0.2272	0.8235

* Considered significant when p-value < 0.05.

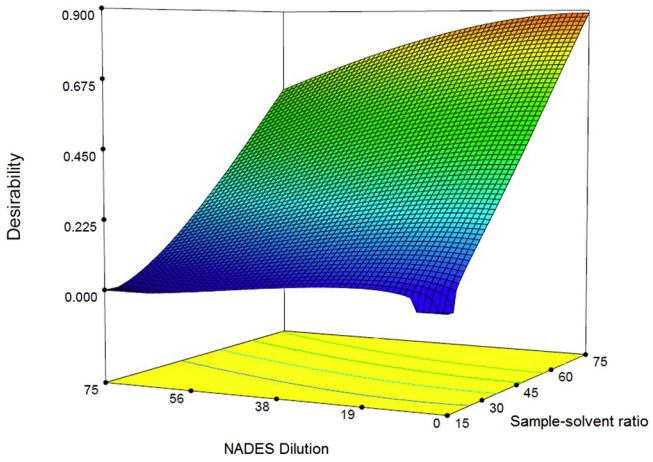

Fig. 1. Response surface plot (Desirability function) for extraction conditions when optimizing NADES dilution and Sample-solvent ratio. The time was kept constant at 42 min (optimal value).

The models were expressed by the following Eqs. (3)–(5)

$$Caf = -0.13 + 0.01\ R + 3.46e-3\ D - 5.62e-5\ R^2 - 3.83e-5\ D^2 \quad (3)$$

$$Fer = -0.76 + 0.02\ R + 7.66e-3\ D - 1.38e-4\ R^2 - 8.41e-5\ D^2 \quad (4)$$

$$Lut = 2.76 + 0.17\ R - 0.06\ D \quad (5)$$

where R: plant-solvent ratio; D: LGH dilution.

The p value showed that at 95% confidence level, the sample/solvent ratio and LGH dilution were significant for the three responses tested whereas the ultrasound time was not significant for them (Eqs. (3)–(5)).

Desirability function was used for the simultaneous optimization of extraction conditions. Thus, target criteria for the analysis of the three selected responses (Caf, Fer and Lut), was to maximize their chromatographic area according to the *L. cuneifolia* phenolic composition. As shown in Fig. 1 the desirability function increases with plant-solvent ratio while decreases with LGH dilution.

The optimal experimental conditions (D = 0.89) for extraction were as follows; plant-solvent ratio of 75 mg mL^{-1}, LGH without dilution and ultrasound time of 42 min. In order to validate the results, three additional experiments at the optimal conditions were conducted. The optimal extraction parameters were thus confirmed.

It has to be pointed out, that the use of chemometrical optimization approaches contribute to “greening” method development since they reduce the experiment number (decreasing solvent and waste amounts, energy and time consumption).

Penalty Points for the optimized methodology were assessed, reaching a total of 4.01 (Table 1). Comparing with the non-conventional techniques previously evaluated the proposed approach showed total PPs similar to those obtained with water demonstrating its sustainability. Thus, an evaluation of the extraction efficiency is necessary.

3.3. HPLC analysis and comparison with traditional solvents

A chromatographic procedure was developed for the analysis of representative phenolic compounds from *L. cuneifolia* (Caf, Fer, Ros, Cat, Cin, Tyr, Nar, Api, Quer, Lut, NDGA and Rut) by means of the one-at a time optimization procedure. The following variables were evaluated: mobile phase composition and gradient, temperature and wavelength detection. Phenolic compounds were separated in 20 min and the optimal conditions are described in Section 2.6.

Analytical figure of merits of standard solutions in LGH are shown in Table 4. For all target compounds, the correlation coefficients of

Table 4
Analytical figure of merit for phenolic compounds in LGH.

Phenolic compound	Retention time (min)	Calibration curve	R^2	LOD[a]	LOQ[a]
Tyr	5.323	y = 0.0381x − 0.0211	0.999	0.071	0.236
Cat	5.947	y = 0,0253x + 0,0053	0.994	0.079	0.263
Cin	12.390	y = 0.4627x − 0.389	0.999	0.003	0.011
Nar	15.307	y = 0.2191x − 0.1884	0,999	0.002	0.008
Caf	6.857	y = 0.2437x + 0.0043	0.998	0.011	0.038
Fer	9.273	y = 0.5662x − 0.8642	0.997	0.004	0.013
Ros	10.837	y = 0.1122x − 0.1914	0.990	0.016	0.053
Api	15.850	y = 0.1445x − 0.1242	0.989	0.002	0.005
Rut	8.463	y = 0,0822x − 0,0573	0.991	0.011	0.037
Quer	13.447	y = 0.0132x + 0.0067	0.987	0.098	0.328
Lut	13.793	y = 0.1428x − 0.2661	0.994	0.009	0.032
NDGA	17.327	y = 0.0316x − 0.0374	0.996	0.041	0.137

[a] LOD and LOQ expressed in μg mL^{-1}.

calibration equations were > 0.987. All of them showed a linear range from the LOQ to 50 μg mL^{-1} at least. The limits of detection (LODs) and quantification (LOQs) were evaluated on the basis of signal-to-noise ratios (S/N) of 3 and 10, obtained respectively.

In order to assess the performance of the HPLC-DAD procedure with the selected conditions, some analytical parameters were evaluated for LGH, MeOH and H_2O as solvent media for the analytes under study. Chromatographic behavior in terms of retention times for LGH was comparable with those for MEOH and H_2O. These results demonstrate the chromatographic compatibility of the eutectic solvent for the analysis of phenolic compounds. Fig. 2 shows the chromatograms for phenolic compounds in LGH, MeOH and H_2O at 280 nm.

In order to determine the matrix effect over each analyte response, calibration curves from a spiked matrix and spiked pure solvent samples were created. Thus, calibration curves from spiked matrix and spiked pure solvent samples were created for each analyte. The percentage of the quotient of the slopes (b) in the spiked and solvent samples was used as an indicator of matrix effect, which were calculated as shown in Eq. (6).

$$Matrix\ effect = 100 - \left[\left(\frac{b_{spiked}}{b_{solvent}}\right)100\right] \quad (6)$$

Matrix effect for the phenolic compounds under study was lower than 20% for all cases. Thus external calibration was chosen. With the aim to estimate the robustness, intra-day repeatability and inter-day reproducibility were evaluated in spiked samples. Samples were fortified before the extraction procedure (5, 25 and 50 μg mL^{-1}). Three extractions of the same spiked sample were carried out before the chromatographic detection. The intraday % RSDs, were between 0.19 and 10.89. The interday values were 2.24 and 10.90. This variability is attributed to matrix effects.

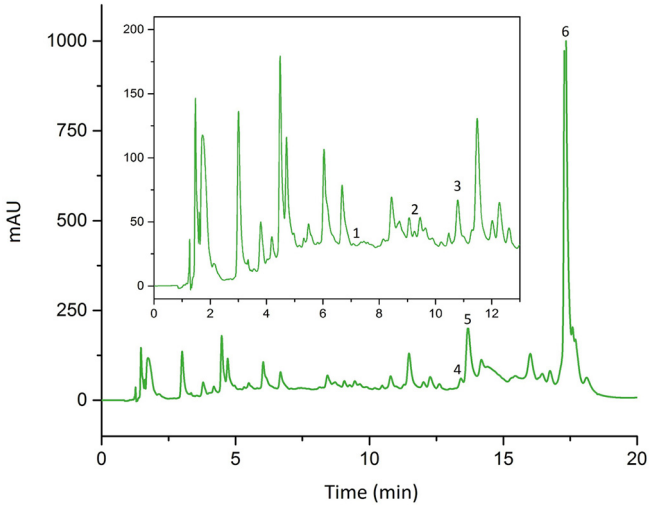

Fig. 2. HPLC-DAD chromatograms of phenolic standards (5 μg g^{-1}) in LGH, MeOH and H_2O at 280 nm. Tyrosol (1), catechin (2), caffeic acid (3), rutin (4), trans-ferulic acid (5), rosmarinic acid (6), cinnamic acid (7), quercetin (8), luteolin (9), naringenin (10), apigenin (11), NDGA (12).

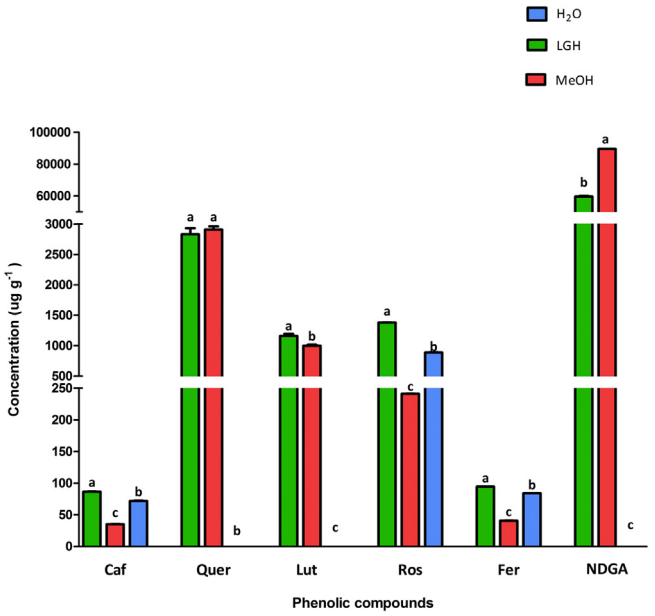

Fig. 3. HPLC-DAD chromatogram of *L. cuneifolia* extract mediated by LGH at 280 nm. Caffeic acid (1), trans-ferulic acid (2), rosmarinic acid (3), quercetin (4), luteolin (5), NDGA (6).

In this work, the optimized extraction procedure mediated by NADES was applied for the determination of phenolic compounds from *Larrea cuneifolia* extract. Among the twelve phenolics studied NDGA, Caf, Fer, Ros, Quer and Lut were detected in the extract (Fig. 3). NDGA was found at the highest concentration, according to other reports this is the most representative compound of *Larrea* species [25–27]. There is scarce information concerning the phenolic composition of *L. cuneifolia* [28–30]. Valesi and co-workers [30] identified the presence of methyl ethers of quercetin and kaempferol in 80% aqueous methanol extract of this plant. Considering the great potential of this medicinal plant, further studies are needed to achieve the complete analytical characterization of *L. cuneifolia*, including derivative phenolic compounds. In other *Larrea* species (*L. divaricata*), Palacio et al. [31] identified NDGA, quercetin and ferulic acid. Our group also reported in water extracts of *L. divaricata*, naringenin, luteolin, quercetin, cinnamic, vanillic and caffeic acids [8].

As previously mentioned, for the extraction of phytochemicals from plants, the most commonly used solvents are methanol, ethanol, hexane, chloroform and water. Thus, the concentration of phenolic compounds extracted by LGH was compared with traditional solvents H_2O and MeOH (Fig. 4). The proposed green solvent presented a highly satisfactory performance. For water-soluble compounds as Caf and Fer, LGH showed an extraction yield better than H_2O. In the case of Quer and Lut, poorly water soluble compounds, LGH showed extraction ability comparable to methanol.

As it has already been stated, green metrics of the proposed approach mediated by LGH are highly satisfactory (Table 1). When comparing the three solvents for the methodology carried out in the present study, water and LGH present similar Green Certificate values. Nevertheless, LGH reveals important advantages in phenolics extraction yields.

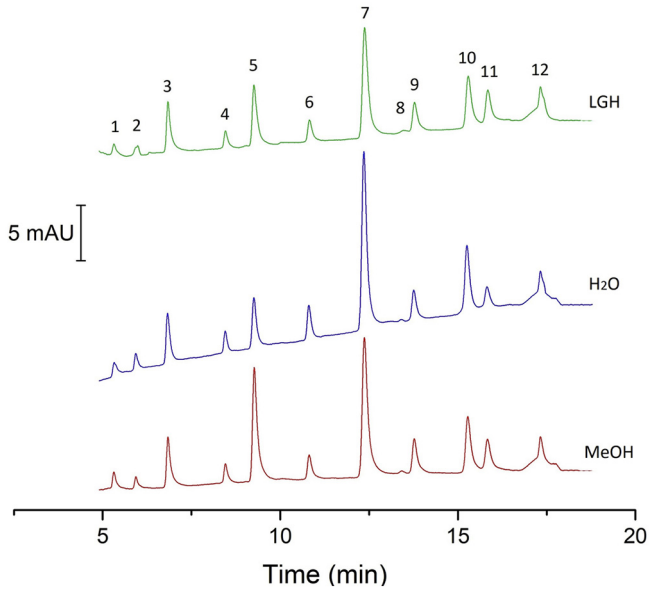

Fig. 4. Concentration of phenolic compounds (μg g^{-1}) in *Larrea cuneifolia* obtained with different extraction solvents. Error bars indicate the SD. Different letters indicate significant differences.

4. Conclusions

The concept of green development has definitely changed the way of thinking processes and methods. In recent years green metrics have been introduced as a valuable tool for evaluate the sustainability of analytical procedures. However, their application as an additional criterion for method selection is still unexploited.

In this work, a NADES mediated UAE extraction was optimized and applied for the recovery of phenolics from *L. cuneifolia*. Indeed, the methodology was evaluated according to the Green Certificate achieving low values of PPs demonstrating its sustainability. Furthermore, the extraction efficiency was compared with traditional solvents, showing LGH outstanding extractability for both polar and weak polar phenolics. It has to be pointed out that the chemometrical optimization contributes to the "green concept". Further research are required to estimate more accurately the energy consumption PPs discriminating between micro and macro scales.

In modern analytical chemistry, a near future goal should be introduction of green metrics as a comprehensive approach to evaluate the greenness of analytical methodology.

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