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Research article

Effect of ultrasound treatment on the extraction of antioxidants from *Ardisia compressa* Kunth fruits and identification of phytochemicals by HPLC-ESI-MS

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ABSTRACT

The influence of ultrasound-assisted extraction of phytochemicals from *Ardisia compressa* Kunth on the antioxidant capacity was investigated. The factors evaluated were: ultrasound extraction time (10, 20 and 30 min), ethanol concentration (0, 35, 70 %) and solid/liquid ratio (1:4, 1:8 and 1:12 g mL^{-1}). The L_9 $(3)^3$ array was applied, and the $DPPH^{\bullet}$ scavenging capacity of treatments was evaluated to obtain optimal extraction conditions. Finally, the phytochemicals were characterized by high-performance liquid chromatography electrospray ionization mass spectrometry (HPLC-ESI-MS). Ten minutes of ultrasound extraction using 0 % of ethanol and solid/liquid ratio 1:12 g mL^{-1} were the optimal conditions of extraction. The HPLC-ESI-MS analysis revealed the presence of gluconic acid, quercetin-3-*O*-glucoside, isorhamnetin-3-*O*-rutinoside, demethylligstroside, ponicidin, 4-caffeoyl-quinic acid, rosmarinic acid, and galloyl-hexoside. The optimal ultrasound-assisted extraction conditions were defined by applying the Taguchi methodology. The phytochemicals identified in *A. compressa* fruits suggest its use as a potential source of bioactive compounds.

1. Introduction

Mexico is one of the countries with the highest plant diversity worldwide. Particularly, the genus *Ardisia* belonging to *Primulaceae* family has remarkable bioactive compounds with pharmaceutical value (Ibarra-Manríquez and Cornejo-Tenorio, 2010; Kobayashi and de Mejía, 2005). Different metabolites of pharmacological interest have been isolated from several *Ardisia* species. Specifically, *Ardisia compressa* Kunth which has been shown to exert different biological activities, such as anticarcinogen on human colon cells (González de Mejía, Chandra, Ramírez-Mares and Wang, 2006), human liver cancer cells (Newell et al., 2010), rat liver (González de Mejía, Ramirez-Mares, Arce-Popoca, Wallig and Villa-Trevino, 2004), anticytotoxic and antigenotoxic effects on rat hepatocytes exposed to benomyl and 1-nitropyrene (González de Mejía & Ramí;rez-Mares, 2002; Ramırez-Mares, Fatell, Villa-Trevino, & de Mejí;a, 1999). These biological activities are thought to be due to *A. compressa* antioxidant properties. *A. compressa* is a shrub found in tropical and subtropical regions of Mexico which produces small fruits with a deep blue/purple color and a delightful sweet and sour flavor. The fruits are used for preparing some artisanal food products as wines, refreshing beverages and jams (Joaquín-Cruz et al., 2015). Most of the phytochemical composition data available for *A. compressa* has been obtained

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from the plants leaves having several bioactive compounds with industrial value as saponins, coumarins and quinones (González de Mejía et al., 2006; Newell et al., 2010). However, to date, there is only one report about physicochemical characteristics, phenolic composition and antioxidant activity of the fruits collected in San Andrés Tuxtla, Veracruz (Joaquín-Cruz et al., 2015).

Nowadays, different novel extraction techniques have been developed for the extraction of different compounds with important biological activities from plant sources (Genovese et al., 2009; Muñiz-Marquez et al., 2013; Wong-Paz et al., 2015 and Mocan et al. 2018). Among the non-conventional extraction methods, the ultrasound is an efficient tool for the extraction of phytochemicals since the ultrasound pass through the liquid medium eliciting the micro-bubbles formation (Muñiz-Marquez et al., 2013). The formed bubbles cause the disruption of cells by impacting them releasing the inner material to the solvent (Wong-Paz et al., 2015). The technique is highly reproducible, easy to manipulate and, reduces the use of solvents and energy (Chemat et al., 2008).

Taguchi methodology is a robust optimization strategy focused to the use of many simultaneous parameters and little experimental trials. The methodology includes the use of orthogonal arrays that allows the reduction of cost and time. In addition, the robust design improves the quality of the tested processes due to the versatile analyzing models. The methodology is considered better than factorial and fractional factorial designs because of the information obtained from the analysis of very few experimental runs (Phadke and Dehnad, 1988).

There are no earlier reports on the use of ultrasound-assisted extraction for the obtention of phytochemicals with antioxidant capacity from *A. compressa*. The objective of this work was to point out the potential use of ultrasound for enhancing the extraction of antioxidant metabolites from *A. compressa* fruits by applying the Taguchi methodology. Also, the characterization of phytochemicals from *A. compressa* fruits by HPLC-ESI-MS was carried out.

2. Materials and methods

2.1. Plant material

The *A. compressa* fruits were manually harvested in Mecatlán, located in Xicotepec de Juárez in the north of the state of Puebla, in Mexico. About 12 kg of fruit were collected from different trees in order to capture as much as possible the biological variability in April of 2018. The plant was identified as *A. compressa* Kunth by Dra. Ernestina Cedillo Portugal corresponding to the number 26072 voucher specimen of a plant previously deposited in the herbarium from Preparatoria Agrícola at Universidad Autónoma Chapingo. Fruits were dehydrated at 60 °C in a convection oven (Memmert, Germany) for 36 h and they were manually ground using a mortar and pestle, to obtain a coarse powder and to separate the seeds from the husk. Later, the samples were pulverized in a homogenizer and sieved to a particle size range between 0.42 – 0.80 mm. The powder was stored until use in the dark at room temperature and low relative moisture.

2.2. Reagents

Ethanol (E5325-17) and methanol (M6125-17) used for extraction were of analytical grade from Jalmek®. 2,2-diphenyl-1picrylhydrazyl (DPPH) radical (D9132-1G) was purchased from Sigma-Aldrich Co®. DPPH• radical solution was prepared freshly every day before measurements.

2.3. Taguchi methodology

The first step in the Taguchi optimization process begins with selection of the appropriate orthogonal array. It is defined on the number of factors and levels for testing (Arvidsson and Gremyr, 2008). The effect of three major factors namely, extraction time (10, 20 and 30 min), ethanol concentration (0, 35 and 70%) and solid/liquid ratio (1:4, 1:8 and 1:12 g mL^{-1}) were investigated. An L_9 (3^3) experimental matrix was obtained showing nine experimental runs derived from the three factors and the three levels (Tables 1 and 2). The antioxidant capacity was evaluated at the end of each experiment. The function higher the better was selected from the three categories (the lower the better, the higher the better and the nominal the best) of quality characteristics in Taguchi methodology. It is expressed as $L(y) = k\ x\ (1/y^2)$ and represented as follow:

$$E[L(y)] = -10^{*}log_{10}\left[(1/n)^{*}\sum\left(1/y_i^2\right)\right] \quad (1)$$

The factor -10 ensures that this ratio measures the inverse of "bad quality" and n represent the number of samples.

For significant factors their percentage of contributions were determined from:

$$P = \frac{SS_i}{SS_T}{*}100\% = \frac{SS_i - MS_i{*}df_i}{SS_T}{*}100\% \quad (2)$$

Where SS_i (square sum of i factor), SS_T (total square sum), MS_i (mean square of i factor) and df_i (degrees of freedom of i factor) were obtained from ANOVA.

2.4. Ultrasound-assisted extraction and validation

The extraction of *A. compressa* fruits was performed by adding 1 g of fruit powder into a test tube and the respective solvent mixture in specified volume, as described in the experimental design (Tables 1 and 2). The tube was then sonicated in the ultrasonic bath (BRANSON 3800, USA) during the time specified in the orthogonal array. After extraction, the supernatant was decanted and refrigerated 24 h at 4 °C for its analysis. All tests were carried out in triplicates.

The optimal extraction conditions of phenolic compounds from *A. compressa* extracts were experimentally validated quantifying the antioxidant capacity. The experimental and predicted values of DPPH• inhibition were compared in order to determine the validity of the model.

2.5. Maceration extraction

The optimal extraction conditions (except sonication time) were reproduced for the extraction of phytochemicals by maceration in order to compare the chromatographic profile with sonicated extracts. Plant material was soaked into distilled water at a solid/liquid ratio of 1:12 g mL^{-1} for 24 h at room temperature (25 °C). Then, the aqueous extracts were centrifuged at 4500 rpm (DLAB; Riverside, CA, USA) and microfiltered (0.45 μm membranes, Merck-Millipore). The extracts were submitted to HPLC-ESI-MS analysis.

2.6. Free-radical scavenging activity

Absorbance of reduced purple solution (fresh DPPH• radical) was measured as following: 100 μL of extract (diluted 1:9 in distilled water) was transferred into test tube (10 mL) and it was mixed with 2.9 mL of DPPH• (60 μM) solution in methanol and, after 30 min in the dark, the absorbance was measured against a methanol blank at 517 nm by a UV Light Spectrophotometer (Model Genesys 10, Thermo Scientific)

Table 1. Selected factors and assigned levels for the Taguchi L_9 orthogonal design.

No.	Factor	Level 1	Level 2	Level 3
1	Time (min)	10	20	30
2	Ethanol (%)	0	35	70
3	Solid/Liquid	1:4	1:8	1:12

Table 2. The L_9 $(3)^3$ Taguchi design experimental matrix for ultrasound assisted extraction of phenolic compounds from *A. compressa* fruits.

Run	Time	% Ethanol	Solid/liquid ratio	DPPH Inhibition (%)	SD
1	1	1	1	80.1	2.9
2	1	2	2	64.0	17.1
3	1	3	3	88.6	2.4
4	2	1	2	87.4	3.7
5	2	2	3	68.6	14.6
6	2	3	1	67.1	7.4
7	3	1	3	82.0	10.1
8	3	2	1	55.1	5.0
9	3	3	2	57.2	4.6

(Molyneux, 2004). Inhibition of the free DPPH radical (% Inhibition) was calculated as follows:

$$\%Inhibition = \frac{Acontrol - Asample}{Ablank} * 100 \quad (3)$$

where *Acontrol* is the absorbance of control reaction (containing all reagents except the tested compound) and *Asample* is the absorbance of the tested compound. All tests were carried out in triplicate.

2.7. HPLC-ESI-MS analysis

The extracts obtained from validation were analyzed by Reversed-Phase High-Performance Liquid Chromatography. It was employed a multi-module equipment that includes a ternary pump (Varian ProStar 230I, USA), autosampler (Varian ProStar 410, USA), and photodiode array detector (PDA) (Varian ProStar 330, USA). Ten microliters of every sample were submitted to a Denali C18 column (150 mm × 4.6 mm, 3.1 μm, Grace, USA) placed into an oven adjusted to 30 °C. The method used was similar to the reported by Aguilar-Zárate et al., (2017). The solvent A was acetic acid 3% and the solvent B was acetonitrile. The flow rate was kept at 0.2 mL/min and the PDA was adjusted to 280 nm for the monitoring of phenolic compounds. The applied gradient was as follow: initial, 3% B; 5–15 min, 16% B linear; 15–45 min, 50% B linear. The column was then washed and reconditioned. Data were processed by software Workstation Multi-Instrument (Version 6.2).

Tandem analysis from HPLC to mass spectrometer were carried out. The liquid chromatograph mass spectrometer was equipped with an electrospray source (Varian 500-MS, USA). All the samples were ionized in negative mode $[M\text{-}H]^-$, nitrogen and helium were used as nebulizing and damping gases. The configuration of ion source was: spray voltage 5.0 kV, capillary voltage 90 V and, temperature 350 °C. Data collection and process were carried out by MS Workstation software (version 6.9) in the *m/z* range 50–2000. Selected precursor ions were fragmented in the order of MS^2 (Aguilar-Zárate et al., 2017).

Table 3. Average DPPH• radical scavenging activity by factor level.

	Level	Means	Estimated parameters	Standard deviation	Standard error
Time	1	77.55	5.32	12.47	1.55
	2	74.38	2.15	11.32	1.48
	3	64.76	-7.47	14.95	1.70
%Ethanol	1	83.15	10.93	3.82	0.86
	2	62.58	-9.65	6.84	1.15
	3	70.95	-1.28	16.05	1.76
Solid/liquid ratio	1	67.43	-4.79	12.46	1.55
	2	69.53	-2.69	15.87	1.75
	3	79.72	7.49	10.18	1.40

2.8. Statistical analysis

The results of antioxidative capacity were analyzed by using the software Statistica 7 (Statsoft, Tulsa, OK). The analysis of variance (ANOVA) were determined based on experimental results. The expected optimum condition showed by the software was experimentally validated and the bias of predicted response from the actual data was calculated. All the experimental trials were performed in triplicate. All the data were shown as the mean ± SD.

3. Results and discussion

3.1. Optimization of antioxidant extraction

The biological properties attributed to natural products are due to their chemistry and/or to synergistic effects. It is a common procedure to measure the total antioxidant capacity of the whole sample (Mihaylova et al., 2015). The DPPH• assay was used in the present work to optimize the ultrasound-assisted extraction of phytochemicals from *A. compressa*. The Taguchi orthogonal array L_9 allowed to experiment the extraction conditions reducing the number of trials to 9 simple and effective experiments. Experimental matrix (Table 2) show the DPPH• reduction capacity of every run revealing their high antioxidant capacity. However, the highest DPPH• inhibition (88%) was obtained at 10 min, 70% of ethanol and 1:12 solid/liquid ratio gmL^{-1}, while the lowest DPPH• inhibition in extraction medium was 55% found in treatment 8 (30 min of sonication, 35% of ethanol and 1:4 solid/liquid ratio gmL^{-1}).

Table 3 shows the average DPPH• inhibition and the estimated parameters for every factor at their corresponding levels. The estimated parameters indicate the mean deviation of every factor level from the overall mean. Also, show the influence of every single factor level on the antioxidant extraction process.

Figure 1 shows the influence of each individual factor on DPPH• inhibition. In the present work, it was observed a decrease in the DPPH• inhibition by increasing the extraction time. It has been reported previously that ultrasonic treatment degraded phenolics from strawberries, whereas degradation decreased by reducing ultrasound exposure time (Herrera and De Castro, 2005). A similar effect was seen in the degradation of anthocyanins due to the increasing ultrasound exposure time (Ravanfar et al., 2015). In the other way, aqueous extracts had higher inhibitory activity against DPPH• radical as compared to ethanolic extracts, which may be attributed to the higher polyphenol content in these extracts (Muñiz-Marquez et al., 2013; Huang et al., 2005).

The relative influence of tested factors expressed in percentage of contribution is depicted in Figure 2. The percentage of contribution for each factor was tested by the ANOVA (Table 4). Ethanol concentration resulted to be most influential factor (50%) on the extraction process followed by time and solid/liquid ratio contributing with 21% and 20%, respectively. An error of 9% was observed. In the Taguchi methodology, the error term not only represent any experimental error. It also shows the influence of two kinds of factors, such as, factors not included in the experiment and uncontrollable factors (Aguilar-Zarate et al., 2014; Van Nostrand, 2002). The value of the error term is not a reflection of the quality of the experiment and does not mean that experiment is bad, or the factors tested are unreliable. There is a rule that mentioned that a factor could be considered as insignificant when its contribution is lower than 10 % (Roy, 2001). In the present study, the error had a contribution of 9 % that could be attributed to uncontrollable/not considered factors such as extraction temperature.

The results obtained experimentally were analyzed applying the higher the better category to obtain the optimal DPPH• radical scavenging activity. The optimum yield was determined at 10 min, 0% of ethanol and 1:12 solid/liquid ratio g mL^{-1} with a prediction of 96 % of DPPH inhibition. However, the DPPH• inhibition experimentally resulted of about 81 % (Table 5). The no use of ethanol, the 10 min of sonication time, and the 1:12 solid/liquid ratio, make the extraction process cost-

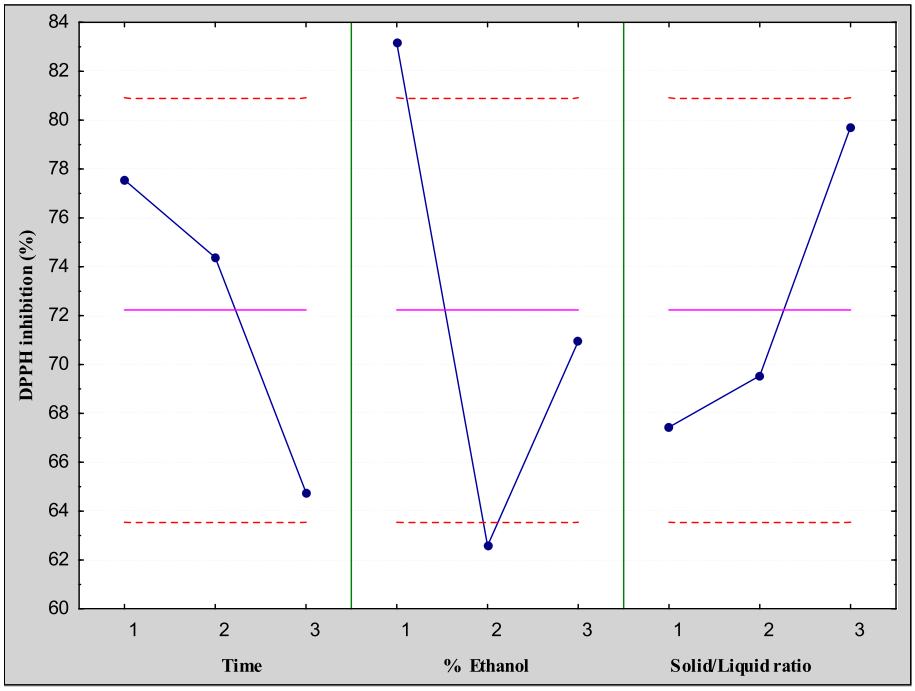

Figure 1. Individual factors performance at different levels.

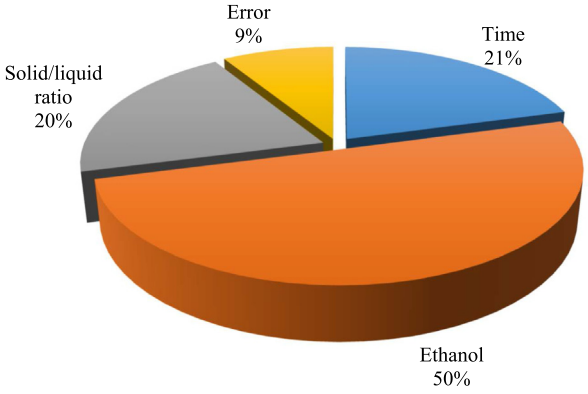

Figure 2. Relative influence of factors on the extraction process.

Table 4. Analysis of variance (ANOVA).

Factors	SS	df	MS	F	p	Percentage (%)
Time	266.12	2	133.06	2.35	0.298531	21
%Ethanol	642.35	2	321.18	5.67	0.149886	50
Solid/liquid ratio	259.10	2	129.55	2.29	0.304161	20
Error	113.26	2	56.63			9
Total	1280.82	8				100

effective compared against the treatments 3, 4 and 7. Despite this, there are three treatments with antioxidant levels above the obtained in experimental validation, the result obtained in the validation step is more reproducible than the results obtained in the experimental matrix since the standard deviation is lower. This assures the reproducibility and the robustness of the validation treatment under all the factors (controllable and uncontrollable). The experimental validation is in good agreement to the expected data with biases below to 15 % (Ravanfar et al., 2015) according to the evaluated equation (Table 5).

Table 5. The predicted values and the experimental results of maximum DPPH• inhibition (%) yield prepared under the optimum condition.

	Level	Effect size	Standard error	Bias (%)
Time	1	5.32	4.34	
%Ethanol	1	10.93	4.34	
Solid/liquid ratio	3	7.49	4.34	
Expected		95.97		
Experimental validation		81.55	0.33	14.16

bias (%) = (predicted value-experimental value)/experimental value *100.

Jindahl & Mohamad (2012) mentioned that higher antioxidant activity is found in fruits compared with leaves of *A. crispa*. They reached 90.2 % and 82.2 % of DPPH inhibition in fruit and leaf methanolic extracts, respectively. The extracts were macerated for 2 h at 40 °C. The use of partially polar solvents, such as methanol, has been a common practice for the extraction of antioxidants. Ramirez-Mares, Sánchez-Burgos & Hernández-Carlos (2010) compared the efficiency of antioxidants extraction by using methanol (polar solvent) and hexane (non-polar solvent). The extraction of phytochemicals with higher antioxidant capacity was obtained in methanolic extracts after two weeks of extraction at room temperature (25 °C).

In the present work, it was demonstrated that the ultrasound-assisted extraction is a helpful tool for eliminating the use of organic solvents, reducing the time of extraction and reaching high levels of antioxidant capacity in extracts from *A. compressa* fruits. So, the optimal extraction conditions involved 10 min of extraction and 0 % of ethanol.

3.2. Characterization of phytochemicals in A. compressa extracts

The phytochemicals from *A. compressa* extracts obtained by optimal conditions of ultrasound-assisted extraction and maceration methods were characterized by HPLC-ESI-MS (Table 6 and Figure 3). A total of 8 of 11 detected chromatographic peaks were identified after the HPLC-ESI-MS analysis, while Peaks 1, 4 and 5 were not identified. The structural characterization of the eight compounds were identified on

Table 6. Identification of polyphenols extracted from *A. compressa* under optimum conditions.

Peak No.	RT	M. W.	$[M-H]^-$ *m/z*	MS^2 ion fragment *m/z*	Tentative assignment	Reference
1	4.33	324	323	301, 265, 245, 197, 183	Unknown	
2	6.60	196	195	177, 159, 130	Gluconic acid	(Felipe et al., 2014)
3	8.00	354	353	173	4-Caffeoylquinic acid	(Jaiswal et al., 2010)
4	9.58	370	369	195, 173, 196	Unknown	
5	14.34	352	351	305, 173; 172	Unknown	
6	15.44	332	331	331, 169	Galloyl-hexoside	(Aguilar-Zárate et al., 2017)
7	21.25	360	359	318, 314, 239, 197	Rosmarinic acid	(Zhang et al., 2012)
8	27.85	510	509	356, 355, 346, 329, 314, 303	Demethylligstroside	(Gentile and Uccella, 2014)
9	30.01	362	361	281, 237, 201	Ponicidin	(Liu et al., 2010)
10	35.12	464	463	317, 316, 302, 301, 300	Quercetin-3-*O*-glucoside	(Felipe et al., 2014)
11	36.36	624	623	316, 315, 314, 300, 299, 271, 255, 244	Isorhamnetin-3-*O*-rutinoside	(Chen et al., 2015)

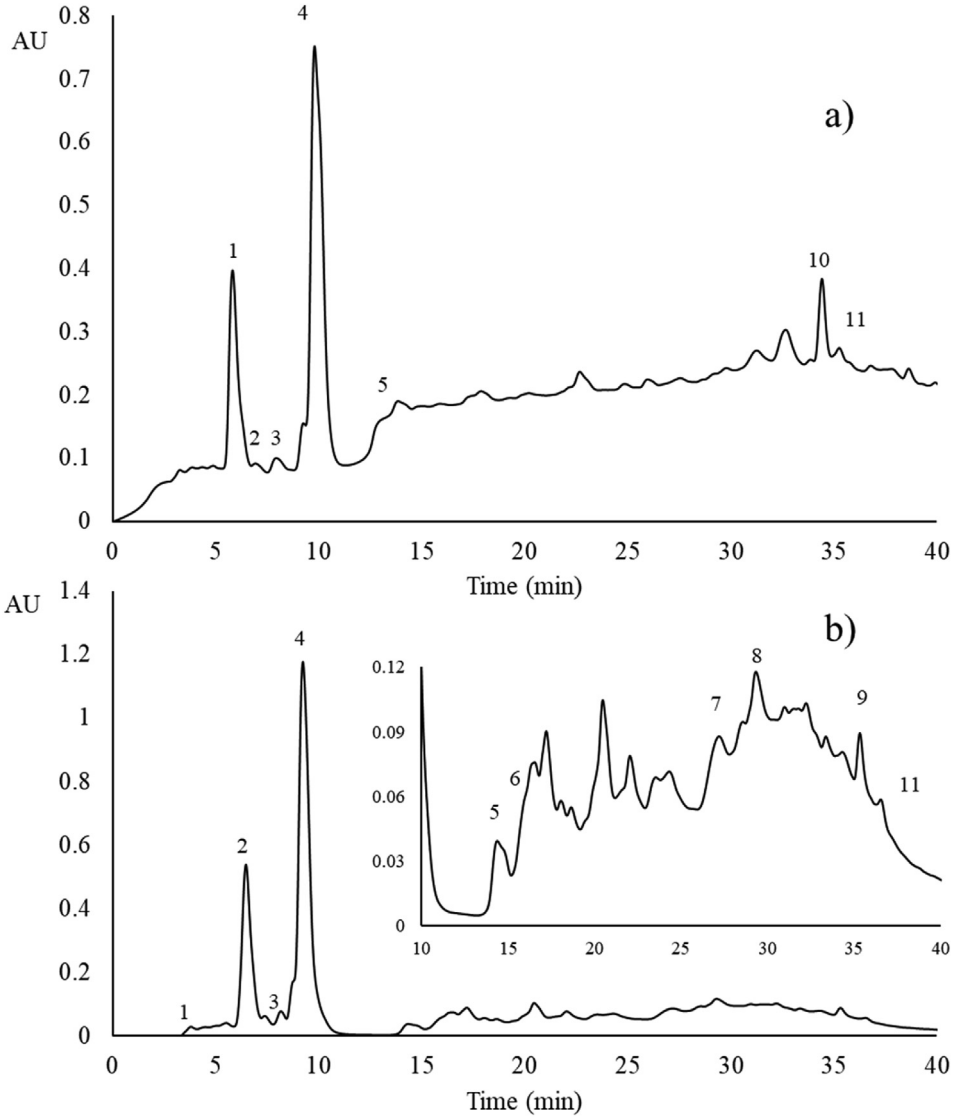

Figure 3. HPLC chromatograms of extracted phytochemicals by a) maceration and b) optimal conditions of ultrasound-assisted extraction.

the basis of their mass spectra using the fragmentation patterns. Thus, the tentative identification of the compounds is summarized in Table 6, where the compounds are numbered according to the retention times in the total ion chromatograms. Several important compounds have been isolated and identified from *A. compressa* extracts. Most of them are polyphenolic compounds as flavonoids particularly as glycosylated flavonoids. It includes the presence 3-*O*-glucosides (quercetin 3-*O*-glucoside, Peak 10) and 3-*O*-rutinosides (Isorhamnetin-3-*O*-rutinoside, Peak 11) (Joaquín-Cruz et al., 2015). Flavonoids are commonly found in fruits, vegetables and herbs and they are known to have potential antioxidant properties and probable roles in preventing oxidative stress associated diseases (Wong-Paz et al., 2017). However, the total number

of sugar moieties, their position, and structure influences the antioxidant activity of such flavonoids (Haminiuk et al., 2012). In addition, the presence of demethylligstroside (Peak 8), belonging to the group of secoiridoids produced by the secondary metabolism of monoterpenes, was shown (Di Donna et al., 2007). Other of the phenolic compounds determined were the diterpenes, as ponicidin (Peak 9) which are phytochemicals known for their diverse biological activities highlighting their ability to counteract oxidative stress (Islam et al., 2016). On the other hand, chlorogenic acids as caffeoylquinic acids (4-caffeoylquinic acid, Peak 3) were identified. The chlorogenic acids are a family of esters formed between quinic acid and certain *trans*-cinnamic acid which are part of a subgroup of polyphenols and they are known to be the most active antioxidant compounds in coffee (Clifford, 1999; Moeenfard et al., 2014). Also, 4-caffeoylquinic acid has been proposed as an antioxidative marker in mulberry (*Morus alba* L.) leaves (Ganzon et al., 2018). Other phenolic compounds such as phenolic acids (rosmarinic acid, Peak 7), which are widely distributed in many plants, including those for dietary applications such as fruits, vegetables and some herbs, were determined and they also, contributed to the antioxidative properties of *A. compressa* extracts (Alfieri and Mann, 2015; Howes et al., 2018). Also, we identify the presence of another phenolic classes as gallotannins, particularly was revealed the presence galloyl-hexoside (Peak 6). The galloyl glucosides are plant polyphenolics that displays various important, diverse and pharmacological activities such as antioxidant and free radical scavenging (Ambika et al., 2014; Hsu et al., 2005; Piao et al., 2009). It is important to mention that all compounds, except, quercertin-3-*O*-glucoside were determined using ultrasound method in comparison to the isolation by maceration.

On the other hand, an acid sugar (gluconic acid, Peak 2) were identified. The gluconic acid is a weak acid which belongs to the aldonic acid family and it is abundantly available in plants, fruits and other foodstuffs. The gluconic acid and its derivatives have an increasing interest in food, pharmaceutical, textile and building industries because they have diverse uses such as additives, preservatives and supplements (Varzakas et al., 2016). Recent research has focused on the antioxidant properties of some gluconic acid derivatives as D-glucono-δ-lactone and sodium-D-gluconate (Cañete-Rodríguez et al., 2016; Ramachandran et al., 2006).

Three unknown compounds were detected by the ESI-MS^2 analysis. They were peaks 1, 4 and 5. The three peaks were detected in both extraction methods (maceration and ultrasound). Peak 4 is the major compound identified in samples. Despite the compounds were not identified in revised literature, the fragments *m/z* 197 [caffeic acid-H+18]$^-$ and *m/z* 173 [quinic acid-H-H_2O]$^-$ suggests the presence of caffeic acid derivatives (Nuengchamnong et al., 2009).

The aqueous extracts of *A. compressa* leaves obtained from Michoacán, Mexico, revealed the presence of gallic acid, proanthocyanidins, epigallocatechin gallate, flavanols or flavanones, quinone derivatives, ardisin and kaempferol (Chandra & González-De-Mejia, 2004). Although the phytochemical profile of *A. compressa* was already analyzed by Joaquin-Cruz et al. (2015) information about the chemical compounds of fruits collected from other regions is still sparse. They show the presence of anthocyanins and up to twenty non-anthocyanins polyphenols in fruits collected from San Andrés Tuxtla, Veracruz, which the most important ones were quercetin glycosides, while the less abundant were phenolic acid derivatives. Only isorhamnetin-3-*O*-rutinoside and quercetin 3-*O*-glucoside were detected in both works showing the influence of cultivated zone in the phytochemical profile.

In fact, climatic condition, soil composition and other factors are reported to influences the synthesis of the plant metabolites (Aliyu et al., 2016). Consequently, the phytochemical content of the plants may vary from place to place as we have shown. To our knowledge, this is the first study on the identification of compounds of *A. compressa* fruits harvested from the north of the state of Puebla by HPLC-MS analysis. This work demonstrated the potential value for *A. compressa* fruits as functional food and for medicinal applications.

4. Conclusion

The combination of Taguchi methodology and ultrasound-assisted extraction allowed to enhance the antioxidant activity of extracts obtained from *A. compressa* by finding the optimal extraction conditions. The high antioxidant capacity, showed by measuring the DPPH$^•$ free-radical scavenging assay, is owing to the variety of bioactive phenolic compounds characterized from its fruits using HPLC-MS technique. *A. compressa* fruits represents a rich source of biologically active compounds such as flavonoids, phenol-conjugated glucosides, diterpenes, gallotannins and chlorogenic acids which may be responsible for the potential health benefits.

Declarations

Author contribution statement

Alma Vázquez-Sánchez: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Pedro Aguilar-Zárate, Guillermo Martinez-Ávila: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Diana Muñiz rquez, Jorge Wong-Paz, Juan Ascacio-Valdés: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Romeo Rojas: Analyzed and interpreted the data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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