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ORIGINAL PAPER

Purple corn (*Zea mays* L.) pericarp hydroalcoholic extracts obtained by conventional processes at atmospheric pressure and by processes at high pressure

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Abstract
Extracts of Peruvian purple corn pericarp (*Zea mays* L.) were obtained: (1) via supercritical fluid extraction with CO_2 as solvent and EtOH-H2O (70:30, v/v) as co-solvent, (2) via pressurized liquid extraction (PLE) with EtOH-H_2O (70:30, v/v) and, (3) via two conventional extraction processes, stirred vessel and Soxhlet. The extraction yields and extract compositions were compared to each other. The parameters measured were the point-to-point extraction yield, the global extraction yield and composition of extracts regarding contents of total phenolics, total flavonoids, total anthocyanins and specific anthocyanins such as cyanidin-3-glucoside, peonidin-3-glucoside and pelargonidin-3-glucoside. Antioxidant activity by DPPH and in vitro antiproliferative activity were also evaluated considering seven cancer cell lines. High yields and higher contents of phenolic compounds, accompanied by a high antioxidant activity at 50 °C, were obtained for supercritical extraction at 60 °C. Antioxidant activity showed good correlation with the content of phenolic compounds, but there was no antiproliferative activity.

Keywords Purple corn pericarp · Supercritical extraction · Phenolic compounds · Anthocyanins · Antioxidant activity · Antiproliferative activity

Introduction

Anthocyanins belong to the class of flavonoids, being responsible for the purple, blue and red colors. Flavonoids are natural compounds found in a wide variety of plant foods. They belong to the class of polyphenols and have antioxidant activity. As natural coloring, anthocyanins have wide application in the food, pharmaceutical and cosmetic industries (Reynertson et al. 2006; Castañeda-Ovando et al. 2009). Potential risks associated with the use of synthetic products, combined with restrictive legislative actions, led to the increase of consumption of natural products (Meng et al. 2012; Navas et al. 2012).

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The Peruvian purple corn (*Zea mays* L., *maiz morado*, in Spanish) is characterized by high contents of anthocyanins. For a long time, its extracts were used as coloring agents for food and beverages. The refreshing traditional drink named "chicha morada" is prepared by immersing the cob into boiling water (Pedreschi and Cisneros-Zevallos 2006). The main anthocyanins found were cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside (Jing et al. 2007; Ramos-Escudero et al. 2012; Monroy et al. 2016a, b).

Studies have shown that phenolic compounds, especially anthocyanins, have beneficial effect on health when consumed frequently, preventing cell degeneration or mutation and thus the appearance of diseases such as heart failure, hypertension, obesity, colon, esophagus, lung, liver, breast and skin cancer, cerebrovascular disease, aneurism rupture and renal injury. Moreover, they have anticarcinogenic properties (Jing et al. 2008; Wang and Stoner 2008; Long et al. 2013), antioxidant activity (Lopez-Martinez et al. 2009; Yang and Zhai 2010), anti-inflammatory activity (Lopez-Martinez et al. 2009) and facile reduction of trigeminal-associated pain in various pathological conditions (Magni et al. 2018).

Recently, several authors (Da Porto et al. 2014b; Garmus et al. 2015; Monroy et al. 2017) studied different techniques to obtain concentrated extracts of phenolic compounds. Although the extraction with supercritical carbon dioxide ($scCO_2$) is considered a promising alternative for safe and sustainable extraction (Vatai et al. 2009; Cavalcanti et al. 2011), the polar nature of many bioactive compounds of interest such as polyphenols and anthocyanins requires addition of a co-solvent (polar) to $scCO_2$ (nonpolar) to increase the affinity (Murga et al. 2000) and solubility of polar compounds, resulting in higher yield (Park et al. 2007; Zarena et al. 2012). Water (polarity = 9.0) and ethanol (polarity = 5.2) have been widely used as co-solvents due to their low cost, besides being "green solvents", with the possibility of direct use in foods and pharmaceutical products. In addition, water–ethanol mixtures as co-solvents proved to be more effective for extracting phenolic compounds (Casas et al. 2009; Da Porto et al. 2014a; Reategui et al. 2014; Solana et al. 2015) with higher yield (Almeida et al. 2013).

Supercritical extraction has considerable advantages over conventional methods: the solvent is easily removed from the solute; little or no organic solvent is used, allowing a quick extraction; and it works at low temperature, favoring the extraction of volatile and thermolabile products (Brunner 2005; Martinez-Correa et al. 2011).

The low stability throughout the extraction process until the storage of the phenolic compounds was studied by different researchers (Qu et al. 2012; Lourith and Kanlayavattanakul 2013; Ardestani et al. 2016; Espada-Bellido et al. 2018; Rodrigues et al. 2018). Besides temperature, the anthocyanin extraction step is also enhanced by other factors such as acidic pH. Acidic (pH of approximately 1.5) conditions are usually preferred for the extraction of anthocyanins (Navas et al. 2012).

The in vitro antiproliferative activity tests, which direct the research to molecules with such potential action on neoplastic cells in culture, are the most widely used (Skehan et al. 1990; Holbeck 2004). This kind of analysis presents conditions to assess various substances in a short time, increasing the possibility to discover new anticancer drugs. Moreover, it is a relatively simple, inexpensive, reproducible technique and provides a potential mechanism of drug action (Suggit and Bibby 2005).

Thus, this study aimed to evaluate the effect of ethanol, water and ethanol/water mixture as co-solvents of $scCO_2$ and as solvents for extraction processes with pressurized liquid (PL), Soxhlet (SOE) and stirred vessel (SVE) to obtain extracts from the purple corn pericarp, through the evaluation of the global extraction yield, phenolics, flavonoids, major anthocyanins, color, antioxidant activity and antiproliferative activity.

Materials and methods

Raw material and reagents

Samples of purple corn pericarp (*Zea mays* L.) were acquired in the Peruvian market. Table 1 shows the characterization of ground raw material. More information on different methodologies and reagents for the characterization of raw material were described by Monroy et al. (2016b).

The following reagents were used in the extraction process: CO_2 purchased from White Martins Industrial (Campinas, Brasil, lot 113C/12) with purity of 99.5%; ethanol (99.8%, v/v) from Êxodo (Brasil lot AE8828RA); ultrapure water obtained from a Milli-Q system (Millipore Corporation, USA). Hydrochloric acid (HCl) (> 99.5% Ecibra, Santo Amaro, Brazil); potassium chloride (KCl) (PA-ACS, Synth, lot 116500, Diadema, São Paulo, Brazil); Sodium acetate (NaC2H3O2) (PA-ACS, Ecibra, lot 17714, Santo Amaro, São Paulo, Brazil); sodium carbonate (99.5%, w/w) and sodium hydroxide (95.0%, w/w) (Êxodo, Brazil); Folin–Ciocalteau from Dinâmica (Brazil); hydrated aluminum chloride (99.0%, w/w) and sodium nitrite (97.0%, w/w) from Ecibra (Brazil); gallic acid (99.0%, w/w) from Vetec (Brazil); (+)—catechin hydrate (98.0%, w/w), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) from Sigma–Aldrich, and 3 standards: cyanidin-3-glycoside, pelargonidin-3-glycoside and peonidin-3-glucoside (> 99,9%, Extrasynthèse, lot 09011533, Sigma).

Table 1 Characterization of pericarp of purple corn (*Zea mays* L.)

Properties	Methodology	Pericarp	References
d_{mg}: Particle diameter (mm)	ASAE	0.50	A.S.A.E (1997)
Volatiles + moisture (%VU)	–	15.4 ± 1.4	A.O.A.C. (1997)
Moisture (%U)	Karl-Fisher	12.1 ± 2.5	A.O.C.S. (1998)
ρ_r: Real densities (g/cm^3)	Helium gas pycnometer	1.37 ± 0.01	–
ρ_a: Apparent densities (g/cm^3)	–	0.386 ± 0.006	Uquiche et al. (2004)
ε: Porosity	$\varepsilon = 1 - (\rho_a/\rho_r)$	0.718 ± 0.006	Rahman et al. (1996)

Supercritical fluid (SFE) and pressurized liquid (PLE) extraction

The SFE process with a CO_2 mixture and co-solvent is shown in Fig. 1. More details can be found in previous studies (Monroy et al. 2016c; Corzzini et al. 2017). The same experimental system used for SFE was used for PLE experiments. In these methods, the same pump was used for pumping EtOH-H_2O (70:30, v/v) as a solvent in PLE or as co-solvent of the SFE, with the non-acidified medium. Previously, the extraction cell was filled with ~ 5 g of ground pericarp and the remaining cell volume was completed with glass balls.

Figure 2 shows the scheme of the processes, using a mixture of EtOH-H_2O (70:30, v/v) as co-solvent for SFE and as a solvent for PLE. The mean flow rate of $scCO_2$ was 1.65 g/min and of EtOH-H_2O (70:30, v/v) was 0.9 mL/min (0.793 g/min), controlled by a high-pressure pump. Experiments were conducted at two temperature levels, 50 °C and 60 °C, and one pressure level of 400 bar for an extraction period of 172 min.

Extractions at atmospheric pressure

Figure 3 shows the extraction scheme used for Soxhlet (SOE) and for stirred vessel (SVE), which were carried out aiming to compare with the extracts obtained via the high pressure processes. Stirred vessel (SV) extraction was conducted in a shaker, adding 2 g of ground material and 25 mL of EtOH-H_2O mixture (0:100, 10:90, 20:80, 30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10 e 100:0 v/v) to 250 mL Erlenmeyer flasks under 150 RPM and at 50 °C for 6 h. In soxhlet extraction, ~ 5 g of the material was used with 150 mL of the hydroalcoholic solvent in different percentages of EtOH:H_2O (0:100, 30:70, 50:50, 70:30 and 100:0, v/v), for 3 h.

Analysis of the extracts

The overall extraction yield (X_0) was used as one of the comparison parameters, which expresses the total mass proportion of extracted solute and mass of the plant matrix used in the extraction process. Total monomeric anthocyanins (TMA) was determined by a differential pH method with readings made at 510 nm and 700 nm (Yang and Zhai

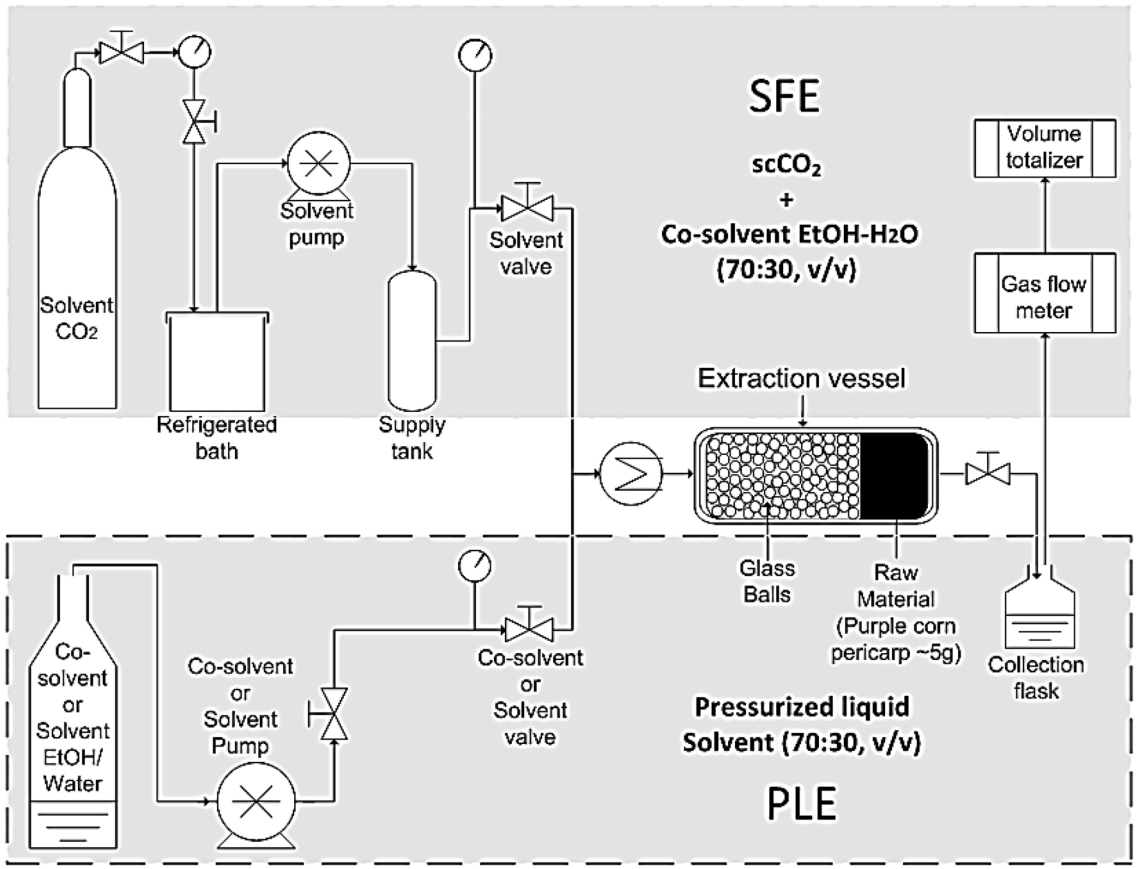

Fig. 1 Schematic diagram of the experimental procedure of supercritical extraction with SFE and PLE

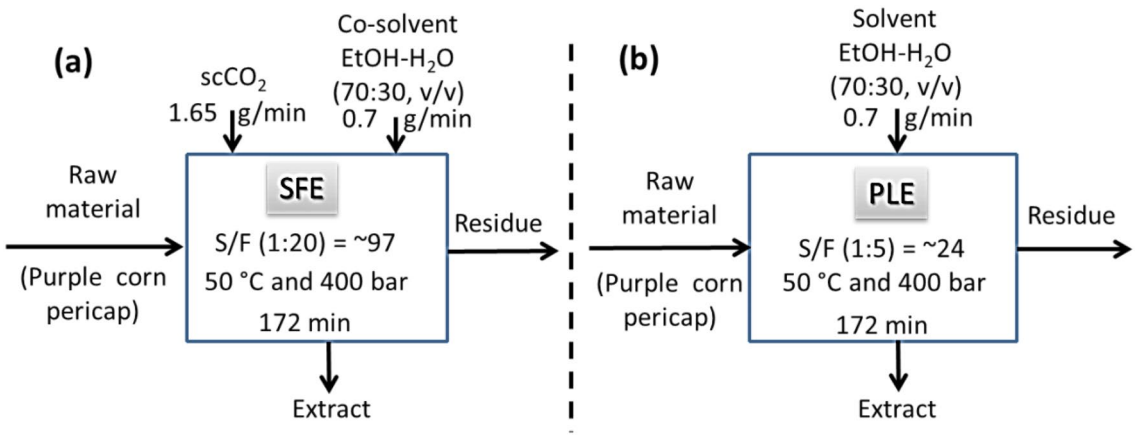

Fig. 2 Extraction process scheme for SFE (**a**) and PLE (**b**): ρ (CO_2) = 1.65 g/L, ρ (EtOH-H_2O) (70:30, v/v) = 0.83 g/mL

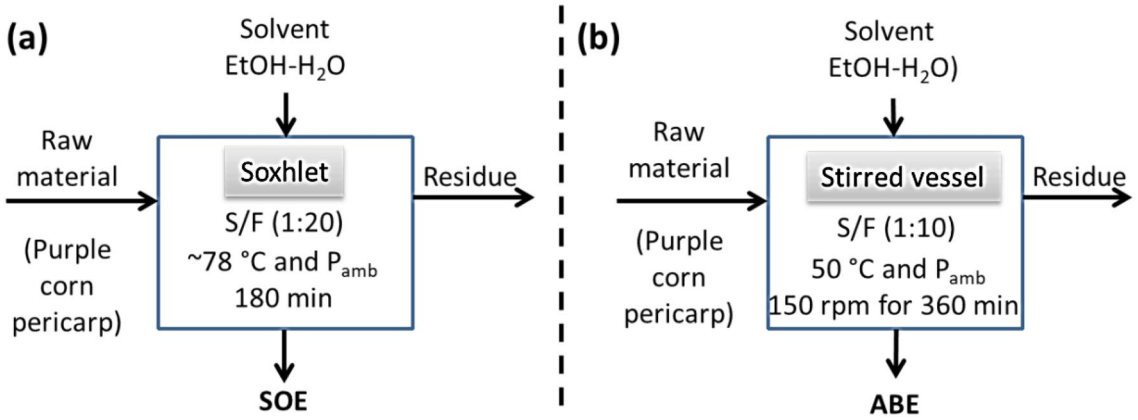

Fig. 3 Scheme of soxhlet extraction (**a**) and (**b**) the stirred vessel process

2010), and absorbance calculated by Eq. (1), and concentration expressed as cyanidin-3-glucoside per gram of extract according to Eq. (2).

$$A = (A_{\lambda max} - A_{700\ nm})_{pH1,0} - (A_{\lambda max} - A_{700\ nm})_{pH4,5} \quad (1)$$

$$TMA = \frac{AxMMxDF}{\varepsilon xb} \quad (2)$$

where TMA = concentration of monomeric anthocyanins (g/L); A = absorbance calculated from Eq. 1; MM = molar mass of cyanidin-3-glucoside (449.2 g.mol^{-1}); = Coefficient of molar absorption of cyanidin-3-glucoside (26.900 L.cm^{-1}.mg^{-1}); DF = dilution factor; b = length of the cuvette path length in the spectrophotometer (cm). Total phenolics (TP) were determined with the Folin-Ciocalteu reagent with reading at 750 nm, expressed as equivalent of Gallic acid (Singleton et al. 1999). The total flavonoids (TF), with reading at 510 nm expressed as equivalents of catechin (Jia et al. 1999). For the antioxidant activity, the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured at 517 nm and expressed as the effective concentration (EC_{50}, μg/mL), which represents the concentration responsible for a 50% decrease in the initial DPPH activity, indicating that the lower the EC_{50} value the greater the antioxidant activity (Mensor et al. 2001). Spectrophotometric readings of the methodologies mentioned were conducted in a spectrophotometer (UV–VIS lambda 40, Perkin Elmer, USA). The analyses were done in triplicate.

High-performance liquid chromatography: HPLC for anthocyanins

Specific anthocyanins, such as cyanidin-3-glucoside (Cy-3-Glu), peonidin-3-glucoside (Pn-3-Glu) and pelargonidin-3-glucoside (Pg-3-Glu), were identified in a LC-DAD Waters Alliance chromatographic system composed of a Waters 2695 pump, Waters 2996 detector and Empower software. Using the column C-18 Waters Nova-Pak (150 × 3.9 mm, 44 μm, with pre-column), two mobile phases were selected,

consisting of a mixture of 10% acetic acid (A) and methanol (B), with a flow rate of 0.5 mL/min. The elution gradient was 0-15 min, 92-75% A and 8-25% B, injection volume was 10 µL in conditions of 30 °C and detection at 510 nm, where anthocyanins were identified by retention times and UV spectra.

In vitro antiproliferative activity tests

The in vitro antiproliferative activity tests were conducted as described by Monks et al. (1991). Seven human tumor cell lines provided by Frederick Ma (National Cancer Institute, Bethesda, MD, EUA) were used, as listed in Table 2. Stock and experimental cultures were cultivated in medium with 5 mL of RPMI 1640 (Roswell Park Memorial Institute) (GIBCO BRL) supplemented with 5% of fetal bovine serum (GIBCO BRL). The penicillin/streptomycin mixture (1000 U/mL: 1000 lg/mL, 1 mL/L of RPMI) was added to the experimental cultures. The cells in 96-wells plates (100 lL cells well1) were exposed to sample concentrations in DMSO/RPMI (0.25, 2.5, 25, 250 lgmL1) at 37 °C and incubated in CO_2 atmosphere at 5% for 48 h, using doxorubicin at concentrations of 0.025; 0.25; 2.5 and 25 µg/mL (100 µL/compartment) in triplicate as positive control.

Table 2 Cell lines used in anticancer activity tests

Cell type	Code	ID[a] ($\times 10^4$ cells/mL)
Lung	NCI-460	4.0
Breast	MCF-7	6.0
Leukemia	K562	6.0
Resistant ovary[b]	NCI-ADR	5.0
Colon	HT-29	4.0
Prostate	PC-O3	5.0
Melanoma	UACC-62	5.0
Ovary	OVCAR-03	7.0
Renal	786-0	4.5

[a]Inoculation density, [b]line that expresses resistant phenotype to multiple drugs

Results and discussion

Extraction kinetics using SFE and PLE

Figure 4 shows the extraction curves represented by the overall yield as a function of the ratio mass solvent and mass sample (S/F) and indicating the facility or difficulty with which the solutes are extracted, using EtOH-H_2O (70:30, v/v) as co-solvent for SFE and as a solvent for PLE, at pressure of 400 bar and temperatures of 50 and 60 °C. A higher yield was observed at the temperature at 60 °C for SFE with 29.1%, followed by the extraction by SFE at 50 °C with 25.4%. The kinetics of the extraction process indicate that the soluble compounds are easily removable and present low mass transfer resistance and high solubility (Martinez-Correa et al. 2011). The kinetic behavior is due to the high density and the polarity of the mixtures, which can lead to solubilization of different compounds in the raw material (Li et al. 2003; Brunner 2005; Wang et al. 2006).

The yield results of 29 and 25% of this study are higher than the 23.8% obtained previously by Monroy et al. (2016a) for purple corn pericarp in a three step sequential extraction with $scCO_2$ in the first step, followed by EtOH and water in the second and third steps, indicating that supercritical extraction with co-solvent is more advantageous than sequential extraction because it uses less solvent and produces higher extract yield.

For other sources of raw materials containing anthocyanic phenolic compounds, as in the research of Cavalcanti

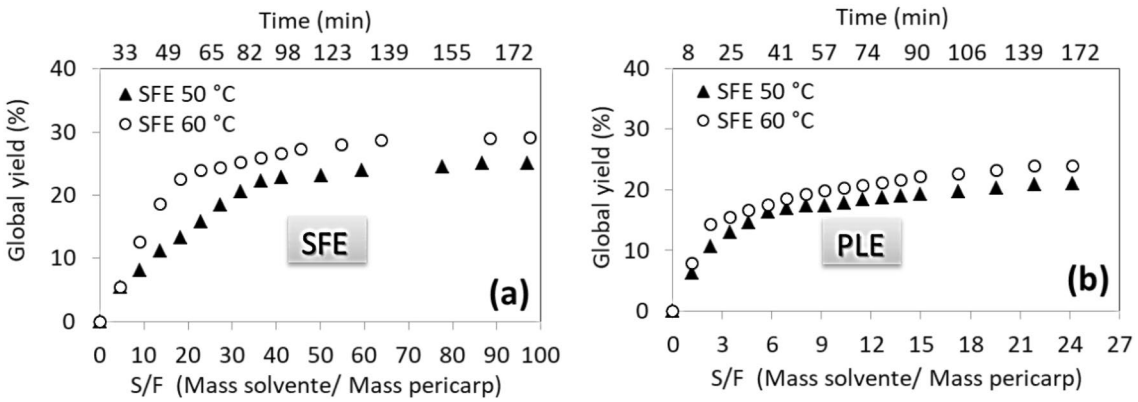

Fig. 4 Kinetic curves for purple corn pericarp extraction (**a**) with CO_2 as solvent (SFE) and EtOH-H_2O (70:30, v/v) as co-solvent, and (**b**) PLE (*pressurized liquid extraction*) with EtOH-H_2O (70:30, v/v) as solvent at 400 bar and 50 °C and 60 °C

et al. (2011) on the supercritical extraction of *jabuticaba* (*Myciaria cauliflora)*, higher yields of anthocyanic compounds were obtained with lower manufacture cost at 323 K and 200 bar using ethanol as co-solvent for $scCO_2$. Serra et al. (2010) studied the extraction of Portuguese cherries, at high pressure with supercritical CO_2 followed by extraction with CO_2 and ethanol at different percentages (90:10, v/v) under the conditions of 50 °C and 25 MPa. In the first step with $scCO_2$, low yields, low concentration of phenolic compounds and low antioxidant activity were obtained compared to extracts obtained in the second step with CO_2 + co-solvent. The extract obtained with CO_2:EtOH (90:10, v/v) showed the highest antioxidant activity (181.4 ± 23.7 µmol TEAC/g).

Figure 5a–d show the results obtained for the extraction via SFE and PLE, in which the kinetics of extraction were observed, with the point yield (Y_0) and contents of total phenolics (TP), of total flavonoids (TF) and of total monomeric anthocyanins (TMA) as a function of the ratio S/F (mass of the solvent in each point and mass of the pericarp of purple corn) and of extraction time (min). High overall yields in the first extracts of SFE were observed, in the range of ratio S/F from 5 to 32 g at 50 °C and 5 g to 18 g at 60 °C. For the extracts obtained by PLE, high yields were observed in the range of S/F from 5 to 8 g at 50 °C and 60 °C with high contents of phenolics, followed by a small decrease of contents of phenolic compounds in the extracts in the subsequent extracts.

Regarding the extraction kinetics of phenolic compounds for different extracts shown in Fig. 5a–d, a similar behavior was observed for all extracts, in which the first extracts have high contents of phenolic compounds followed by the decrease of such contents.

Figure 6 shows the HPLC chromatogram obtained at 510 nm for a sample corresponding to the original extract of Pericarp corn. Figure 6 shows the chromatogram obtained at 510 nm by HPLC for a sample corresponding to the original extract of Pericarp corn, identifying and quantifying three specific anthocyanins, Cy-3-Glu, Pn-3-Glu and Pg-3-Glu, with retention times of 7.512 min, 9.903 min and 11.473 min respectively. These main anthocyanins were also found by several researchers (Jing et al. 2007; Ramos-Escudero et al. 2012; Monroy et al. 2016a, b; Lao et al. 2017; Paucar-Menacho et al. 2017; Lao and Giusti 2018)). Figure 7 shows the behavior of specific anthocyanins content with the extraction times, detecting that Cy-3-Glu is the most abundant with the

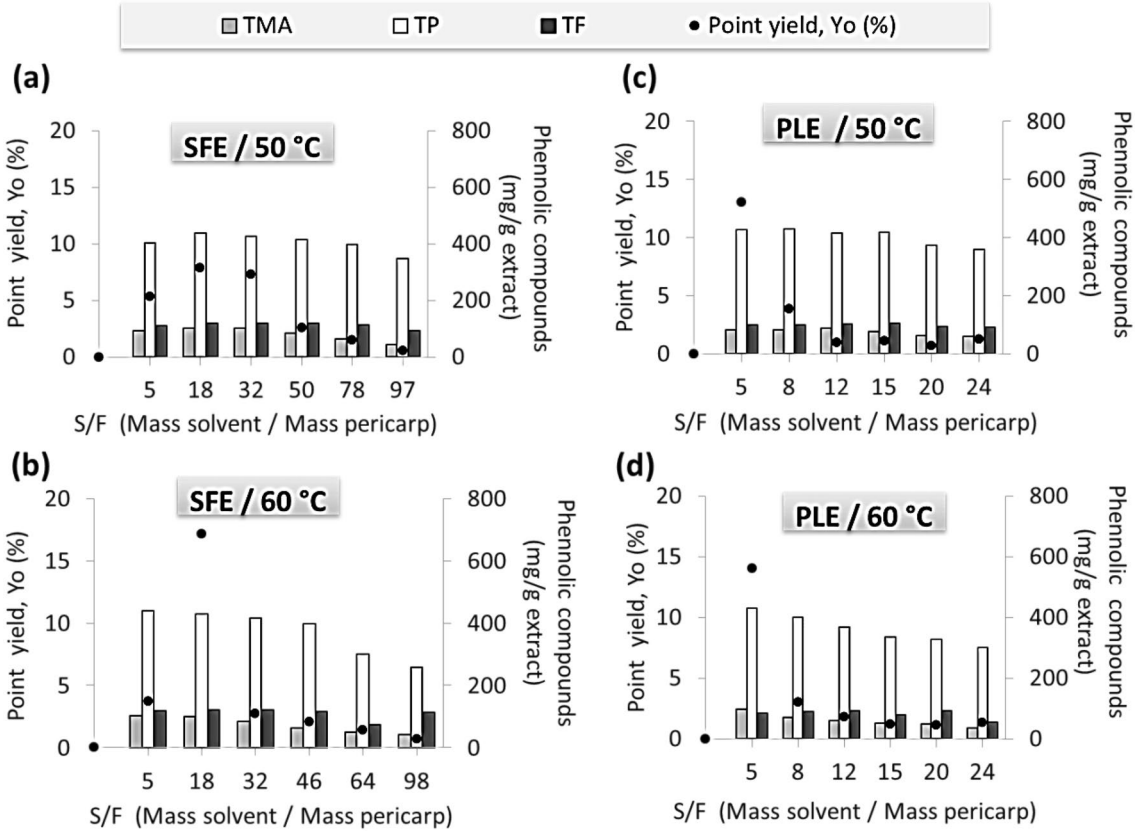

Fig. 5 Point yield of extracts, phenolic compounds (ATM, TP and TF) to SFE (**a**, **b**) and PLE (**c**, **d**) at 50 °C and 60 °C, and constant pressure of 400 bar of extracts of pericarp of purple corn

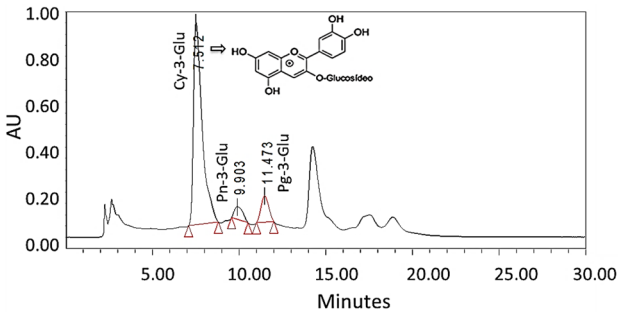

Fig. 6 Chromatograms obtained at 510 nm by HPLC

highest values in the first extracts, obtaining values greater than 40 mg/g extract. The amount of anthocyanin extracted decreases with increasing extraction time or with the ratio of S/F (Mass solvent/Mass pericarp). As shown in Table 3, the contents range from 24.1 to 26.8 mg/g extract for Cy-3-Glu, from 4.3 to 5.4 mg/g extract for Pn-3-Glu and from 4.4 to 5.1 mg/g extract for Pg-3-Glu in different extracts obtained via SFE and PLE.

For Santos et al. (2012) the kinetic behavior in jabuticaba skins extraction using PLE shows that, at the beginning of the extraction procedure, the amount of anthocyanins and total phenolic compounds extracted increases with increasing extraction time. The presence of ethanol in the medium facilitates anthocyanin and especially proanthocyanidin extraction (Canals et al. 2005).

The high content of total phenolic compounds, about 400 mg/g in the SFE and PLE extracts, as shown in Fig. 8a–d, contributed to the high antioxidant activity, with EC50 values less than 50 µg/mL In the study of six different corn varieties from China, Zhu et al. (2014) demonstrated that there were positive correlations ($R^2 = 0.9911$ and $R^2 = 0.9873$) between anthocyanins and elimination activity of superoxide anion radicals or power of reduction, respectively. Othman et al. (2017) evaluated the antioxidant capacity of the banana cultivar 'Nipah' (*Musa acuminate* balbisiana) extracted with different solvents, demonstrating that high phenolic content determines the high antioxidant capacity of the fruit.

Extraction in stirred vessel and in soxhlet

Results of extractions conducted at atmospheric pressure in a stirred vessel (SVE) and soxhlet extractor (SOE) obtained using different proportions of ethanol/water mixture are shown in Fig. 9, which present values of extraction yield, contents of phenolic compounds (TP, TF and TMA) and specific anthocyanins (Cy-3-Glu, Pn-3-Glu e Pg-3-Glu). The SV extracts had high yield in the range of EtOH-H_2O (40:60-70:30, v/v) concentration and high contents of phenolic

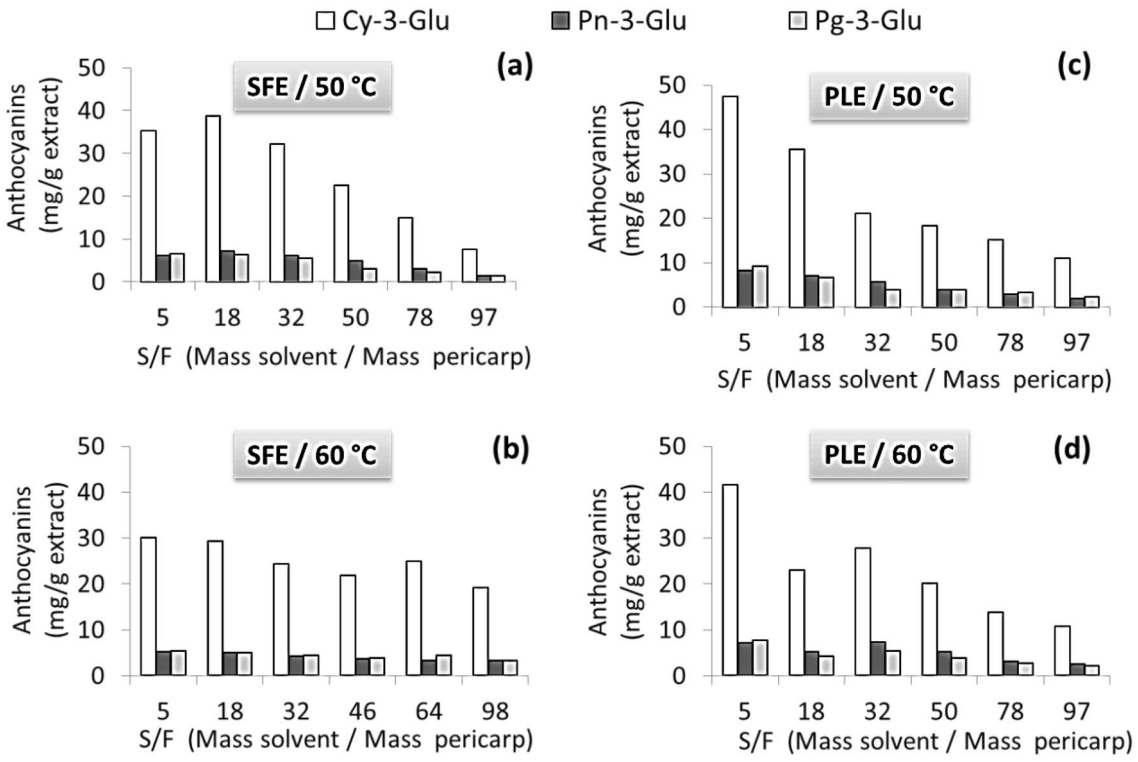

Fig. 7 Specific anthocyanins found in extracts of pericarp of purple corn, from extracts obtained via SFE (**a**, **b**) and PLE (**c**, **d**) at 50 °C and 60 °C

Table 3 Concentration and yields of TP, TF, TMA and AA (EC_{50}/DPPH) of extracts from pericarp of purple corn (*Zea mays* L.) via different extraction methods

Type of extraction	X_0: Overall yield (%)	TP		TF		TMA	
		C1	R1	C2	R2	C3	R3
High pressure extraction							
SFE (50 °C)	25.4	405 ± 14	103 ± 4	113 ± 10	29 ± 3	81 ± 9	21 ± 2
SFE (60 °C)	29.1	373 ± 19	109 ± 5	110 ± 7	32 ± 2	74 ± 6	22 ± 2
PLE (50 °C)	21.7	404 ± 9	88 ± 2	99 ± 9	21 ± 2	75 ± 7	16 ± 2
PLE (60 °C)	24.3	361 ± 16	88 ± 4	82 ± 6	20 ± 1	61 ± 7	15 ± 2
Low pressure extraction							
SOE 70:30	22.3	100 ± 7	22 ± 2	70 ± 7	16 ± 2	22 ± 3	5 ± 1
SVE 70:30	20.4	207 ± 10	42 ± 2	121 ± 8	25 ± 2	34 ± 5	7 ± 1
Type of extraction	AoA	Cy-3-Glu		Pn-3-Glu		Pg-3-Glu	
		C4	R4	C5	R5	C6	R6
High pressure extraction							
SFE (50 °C)	8.1	26.8 ± 5.3	6.8 ± 1.3	5.1 ± 0.4	1.3 ± 0.1	4.4 ± 0.6	1.1 ± 0.1
SFE (60 °C)	8.5	25.7 ± 5.2	7.5 ± 1.5	4.3 ± 0.4	1.3 ± 0.1	4.6 ± 0.8	1.3 ± 0.2
PLE (50 °C)	9.8	26.1 ± 5.6	5.7 ± 1.2	5.2 ± 0.6	1.1 ± 0.1	5.1 ± 0.1	1.1 ± 0
PLE (60 °C)	10.3	24.1 ± 5.5	5.9 ± 1.3	5.4 ± 0.6	1.3 ± 0.1	4.6 ± 0.8	1.1 ± 0.2
Low pressure extraction							
SOE 70:30	15.5	24.9 ± 2.6	5.5 ± 0.6	1.6 ± 0.1	0.4 ± 0	6.2 ± 0.3	1.4 ± 0.1
SVE 70:30	17.2	16.8 ± 0.8	3.4 ± 0.2	2.2 ± 0.3	0.4 ± 0.1	5.6 ± 0.8	1.1 ± 0.2

X_0 extraction yield (%, d.m.), *C1* concentration (mg GAE/g extract), *C2* concentration (mg EC/g extract), *C3* concentration (mg C3G/g extract), *C4* effective concentration EC_{50}/DPPH (μg/mL), *R* yield (mg/g pericarp)

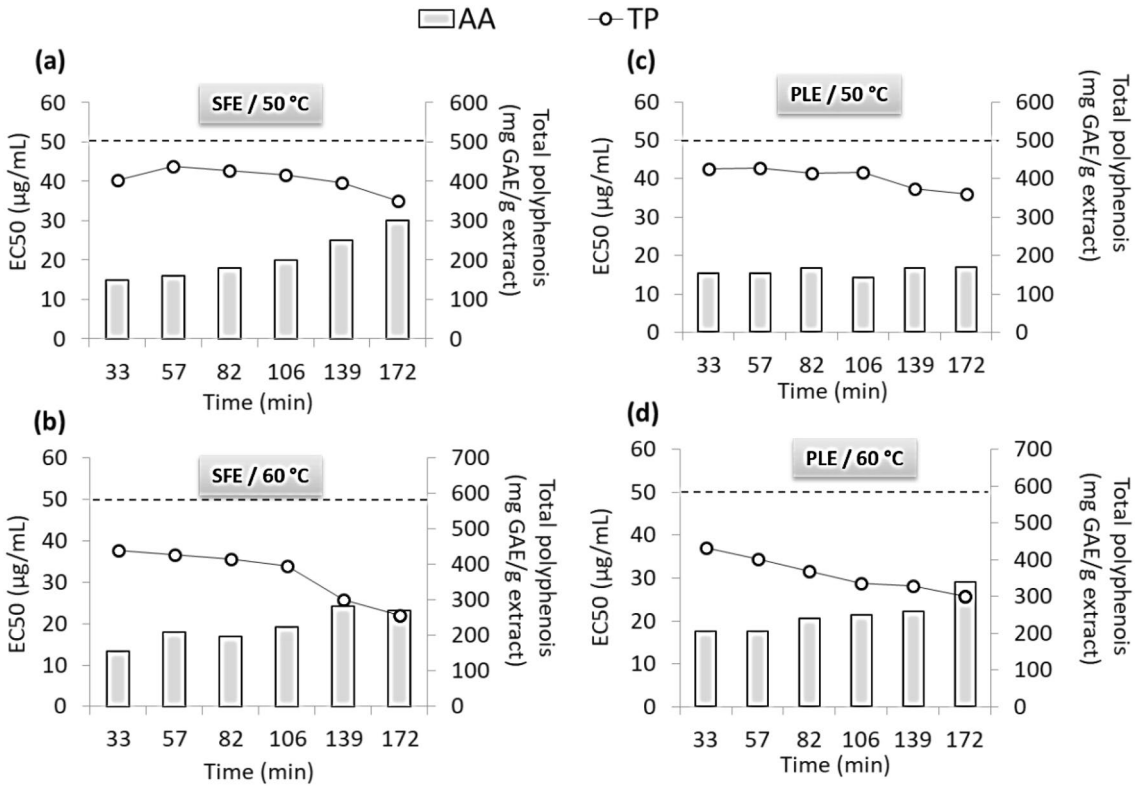

Fig. 8 Comparison of antioxidant activity expressed as EC_{50} and total phenolics (TP) for extracts via SFE (**a**, **b**) and via PLE (**c**, **d**), of the pericarp of purple corn

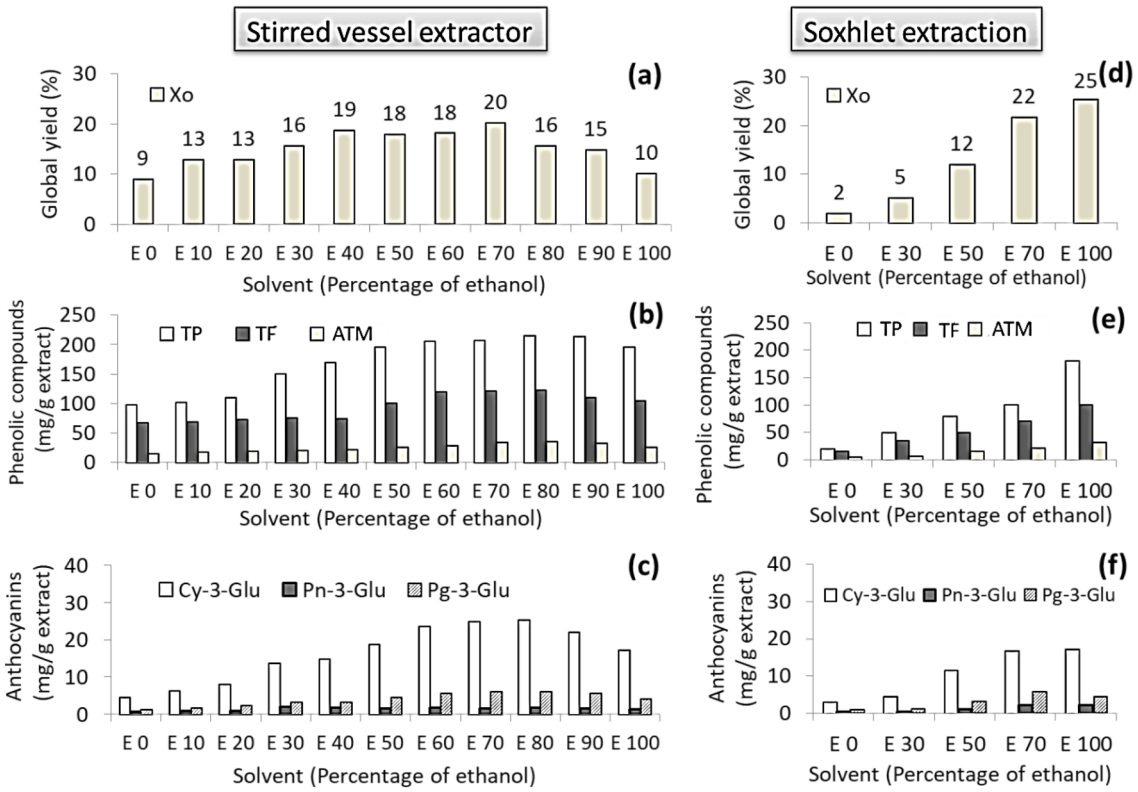

Fig. 9 Global yield of extracts (**a**, **d**), TP, TF and ATM concentrations (**b**, **e**), specific anthocyanins concentration (**c**, **f**) of the extracts obtained via stirred vessel (**a**, **b**, **c**) and via soxhlet (**d**, **e**, **f**) of the pericarp of purple corn

compounds in the range of EtOH-H_2O (70:30-100:0, v/v), while for the specific compounds in the range of EtOH-H_2O (60:40-90:10, v/v). For the extracts obtained via SO using EtOH-H_2O (70:30 e 100:0, v/v), high yields, high contents of phenolic compounds and of specific compounds were obtained.

Ramos-Escudero et al. (2012) evaluated different percentages of methanol:water MeOH:H_2O acidified with HCl at 1% in the conventional extractions, finding high contents of phenolic compounds when the MeOH:H_2O 80:20 mixture was used. Stojiljkovic et al. (2016) obtained extracts at atmospheric pressure of wild apple fruit from Serbia with different solvents; the extracts obtained using ethanol and distilled water as solvents contained the highest amount of polyphenolic compounds and demonstrated the best antioxidant activity.

Comparison between extraction methods

Table 3 shows, for the SFE, PLE, SV and SO extracts, the overall yield (X0), total yield and concentration of phenolics (TP), flavonoids, total monomeric anthocyanins (TMA), cyanidin-3-glucoside (Cy-3-Glu), peonidin-3-glucoside (Pn-3-Glu), pelargonidin-3-glucoside (Pg-3-Glu) and antioxidant activity values expressed as EC50 (μg/mL). Contents of phenolic compounds of SFE and PLE extracts were considered to be the mean of values obtained point-to-point for the kinetics shown in Figs. 5 and 7 at both temperatures. For the extracts obtained via SV and SO, values using EtOH-H_2O (70:30, v/v) as solvent were considered, which were shown in Fig. 9. It is observed that all types of extraction shown in Table 3 have high overall yields between 20 and 29%, but the extracts obtained at high pressure by supercritical or pressurized liquid extraction had higher contents of phenolic compounds and anthocyanins and consequently higher antioxidant activities. However, when Farias-Campomanes et al. (2013) performed grape bagasse extractions at high and low pressures, they found that the highest extraction yields were observed for processes that used conventional extraction techniques; however, the results indicated that, in the case of phenolic compounds recovery from a Pisco bagasse, the SFE process was the most efficient. In the extraction comparison of SFE and PLE from *Asparagus officinalis* L, Solana et al. (2015) demonstrated that the presence of water and ethanol is essential to obtain a phenolic enriched extract with high antioxidant activity. Operating conditions influence the extraction yield, however the phenolic composition of the extract does not vary significantly. Extraction of quinic acid (main phenolic acid) was more efficient by PLE than by SFE and Soxhlet.

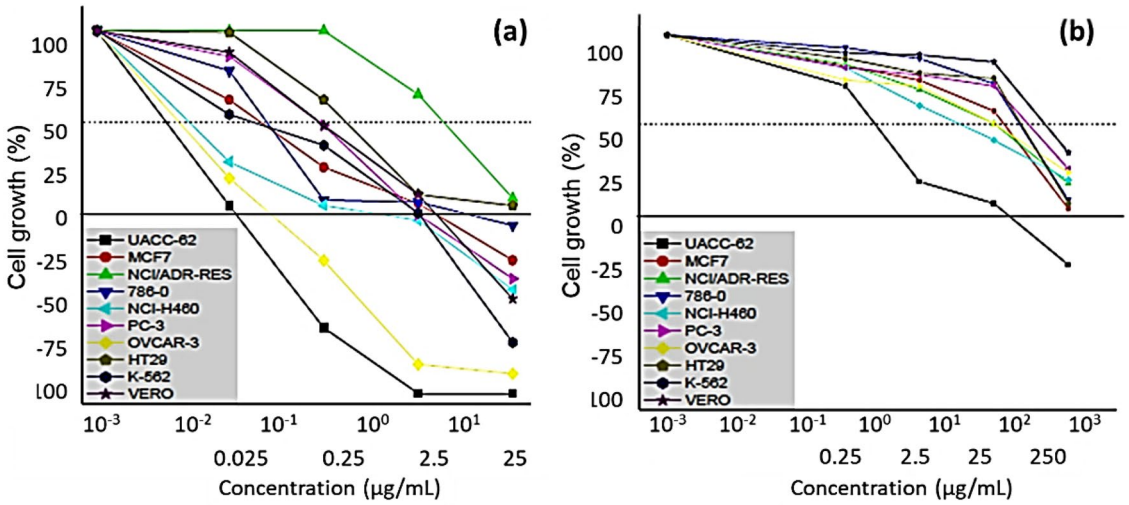

Fig. 10 Concentration-response curve of the chemotherapy drug doxorubicin (**a**) and of the extract obtained via SFE at 50 °C (**b**), relating percentage of growth versus concentration of sample. (UACC-62—melanoma, MCF7—breast, NCI/ADR-RES—ovary resistant to multiple drugs, 786-0—kidney, NCI-H460—lung, PC-3—prostate, OVCAR-3—ovary, HT29—colon, K-562—leukemia e VERO—non-tumor cell line)

In vitro antiproliferative activity of extracts of pericarp of purple corn

Figure 10a shows the action of the chemotherapy drug doxorubicin in human tumor cell culture and relates the percentage of cell growth with chemotherapy drug concentration. Figure 10b shows the extracts of pericarp of purple corn obtained via SFE at 50 °C and 400 bar which were evaluated considering the same cells.

Dose–response analysis of cell growth inhibition by the pericarp extract demonstrated no activation for the lines. Some studies with vegetable extracts rich in anthocyanins have shown antiproliferative activity: the purple potato had in vitro (Madiwale et al. 2011) and in vivo (Lim et al. 2013) antiproliferative activity; the aronia had antiproliferative activity in cervical tumor cell lines, (Rugina et al. 2012), Serra et al. (2010) demonstrated that, in cherry extracts obtained with CO_2:EtOH (90:10, v/v), there was higher antioxidant activity and it was the most effective in inhibiting the growth of human colon cancer cells ($ED5096h = 0.20 \pm 0.02$ mg/mL).

Several studies showed good antiproliferative activity, which may depend on the extraction method and the tumor cell lines used. Long et al. (2013) reported the inhibition of cell proliferation in a tumor line of prostate cancer, through inhibition of the Gap 1 (G1) stage of the cell cycle, Fukamachi et al. (2008) reported inhibition of cell proliferation of breast cancer; the levels of RAS proteins (protein important to control cell multiplication and differentiation) were reduced in tumor cells. Purple corn may have good antiproliferative activity depending on the conditions of extraction and the tumor lines used.

Conclusion

Considering the hydroalcoholic extracts, the general extraction yield and the phenolic content of supercritical and hydroalcoholic extracts were strongly influenced by the use of EtOH-H_2O (70:30, v/v mixture) as co-solvent for SFE and as solvent for PLE. Concentration of phenolic compounds and antioxidant activity of the extracts obtained via PLE were slightly lower than those obtained via SFE. However, because the PLE process is more inexpensive, faster and also has the main advantages of SFE (environmentally friendly at moderate temperatures), it can be considered as a good alternative to extract bioactive compounds of the pericarp of purple corn.

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