Biocatalysis and Agricultural Biotechnology 19 (2019) 101169

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Supercritical CO_2 to recover extracts enriched in antioxidant compounds from beetroot aerial parts

Heloísa Fabian Battistella Lasta, Lucas Lentz, Natália Mezzomo, Sandra Regina Salvador Ferreira*

Chemical and Food Engineering Department, Federal University of Santa Catarina, EQA/UFSC, C.P. 476, CEP 88.040-900, Florianópolis, SC, Brazil

ARTICLE INFO

Keywords:
SFE
Beta vulgaris L.
Extraction methods
Antioxidant potential
Phenolic compounds

ABSTRACT

Beetroot is a well appreciated edible root, widely used for sugar and ethanol production, and source of natural colorant. Its aerial parts, leaves and stems, are frequently discarded as animal feed or manure. Therefore, the recovery of bioactive components from these materials is appealing due to abundance of raw material, its low price and poor valorization, allied with the lack of related studies. Soxhlet, maceration, ultrasound and supercritical fluid extraction (SFE) were compared in terms of process yield and extract quality, particularly related to its total phenolic content (TPC) and antioxidant potential. Extracts were compared with standard antioxidant BHT (butylated hydroxytoluene), and the highest TPC values were obtained by SFE with ethanol:water and ethanol as cosolvents, up to 99 ± 4 mgGAE/$g_{extract}$ and 98 ± 6 mgGAE/$g_{extract}$, for leaves and stems respectively. The bioactive substances extracted from these residues may present a high potential for reused in food or pharmaceutical industries, supporting the closed cycle manufacturing.

1. Introduction

Beta vulgaris L., primarily cultivated for its roots, with high nutritional value, are usually consumed after cooking and one of the most important sugar crops in the world. The beetroot residues outcome from various steps of the processing chain, from harvesting to transportation, and up to industrial by-products. Although several works deal with the valorization of beetroot by-products (Vaccari et al., 2005; Krajnc et al., 2007; Duraisam et al., 2017), minor attention is placed to the aerial parts, consisting of leaves and stems, which are usually discarded at field or before the industrial processing, despite the presence of valuable components (Duraisam et al., 2017; Storck et al., 2013). These aerial parts are used as animal feed or fertilizer, although rarely, due to the high content in calcium, iron, sodium, vitamins A, B_1 and B_2, at levels significantly higher than those of roots (Rana, 2016). This reveals the importance of the development of procedures to recover such valuable substances. Also, according to Ninfali and Angelino (2013), *Beta vulgaris* L. root contains high amount of betalains, a large group of water-soluble pigments, associated with high antioxidant potential (Mikolajczyk-Bator et al., 2016). Besides, phenolic compounds, important antioxidant compounds, were also found by Guiné et al. (2018) in beetroot samples and in this vegetable leaves by Biondo et al. (2014).

The quality of bioactive extracts is dependent on the extraction process, solvent type and raw material characteristics, which must be carefully selected to maximize yield and selectivity (Azmir et al., 2013). Traditional extraction methods, normally applied to recover bioactive extracts, present drawbacks such as high temperature, energy input and process time, combined with low selectivity and use large amounts of solvents, mostly toxics. These problems reduce product quality and are environmentally unacceptable. Then, Supercritical fluid extraction (SFE) is an alternative to recover compounds from natural products, with CO_2 as the most recognized solvent, classified as GRAS (Generally Recognized as Safe) (US-FDA, 2016). Low viscosity and relatively high diffusivity from supercritical solvent provide good transport properties, with fast recovery, compared to conventional methods (Michielin et al., 2009; Herrero et al., 2006).

Besides the recognized importance of the residues from *Beta vulgaris* L., there is a lack of studies related to its leaves and stems, particularly concern to their antioxidant potential. Therefore, the objective of the present work was to obtain extracts from beetroot aerial parts by SFE, with CO_2 and cosolvent, and compare with Soxhlet, maceration and ultrasound extractions using different solvents. Process yield and product quality were evaluated to provide an alternative use from these agroindustrial residues. Also, the various extracts were characterized in volatile fraction by gas chromatography for the identification of compounds of interest.

* Corresponding author.
E-mail address: s.ferreira@ufsc.br (S.R.S. Ferreira).

https://doi.org/10.1016/j.bcab.2019.101169
Received 20 March 2019; Received in revised form 3 May 2019; Accepted 19 May 2019
Available online 21 May 2019
1878-8181/

2. Material and methods

2.1. Vegetable raw material

Beetroot leaves and stems were provided by "*Programa dos Trabalhadores Rurais Sem Terra*" (Chapecó, SC, Brazil), with specimen deposited at *Viveiro Florestal Universitário* from UNOCHAPECO (Chapecó, Santa Catarina, Brazil). The raw materials, collected in November and December of 2015, were separated (leaves and stems), water sanitized and wiped with a paper towel and stored in a domestic freezer at −18 °C. After that, they were dried in a forced air circulation oven (DL-SE, DeLeo) at 45 °C for 10 h for the leaves and 24 h for the stems, reaching moisture and volatiles content of 8.48 g/100 g and 6.62 g/100 g, respectively, evaluated according to 012 IV method (IAL, 2008). Dried materials were grounded in a stainless steel knife mill (DeLeo, Brazil). The mean particle diameters, based on mean size distribution, were 0.258 mm and 0.267 mm, for leaves and stem, respectively. Before use, the samples were packaged and stored at −18 °C (Consul CVU 300, Brazil), under light protection. The analyses of plant raw materials characterization (moisture and volatile content, ash, fat, crude fiber and protein) were carried out at Food Science and Technology Department from Federal University of Santa Catarina following standard protocols IAL (2008).

2.2. Extraction methods

The extraction techniques were divided in two groups: (1) low pressure methods (Soxhlet, maceration and ultrasound-assisted) performed with solvents and standard procedures listed below, and high pressure method (supercritical fluid extraction), using carbon dioxide and cosolvent. The according methods are presented as follow.

2.2.1. Soxhlet extraction (SOX)

Beta vulgaris L. aerial parts were submitted to Soxhlet extraction according to method 032/IV (IAL, 2008). It consisted of refluxing 150 mL of solvent over 5 g of the dried sample for 8 h in a Soxhlet apparatus. The solvents were: water (H_2O); a mixture of water and ethanol 1:1 (v/v) (H_2O–EtOH); ethanol (EtOH) and hexane (Hx), with a polarity index of 9.0, 7.2, 5.2 and 0, respectively (Reichardt, 2003). The assays were performed at least in duplicate at solvent boiling temperature. Residual solvent from crude extracts was removed in a rotary evaporator (Mod. 801 and 802, Fitasom, Brasil). Samples were stored in amber flasks at −18 °C (Freezer 250, Brastemp, Brazil). The yield (*mass of extract*/*mass of raw material*) was presented as an average ± standard deviation.

2.2.2. Maceration (MAC)

Performed at 25 °C, according to Oliveira et al. (2016). Shortly, 5 g of material and 25 mL of solvent were placed in a flask for four days, manually shaken once a day. The solvents, same as from section 2.2.1, were applied separately. Residual solvent was removed, the extracts stored and the yield determined according to section 2.2.1.

2.2.3. Ultrasound-assisted extraction (UAE)

Carried out in duplicate, according to Souza et al. (2016). UAE was performed with 5 g of sample and 150 mL of solvent at room temperature (25 °C), submitted to 7 min of 500 W sonication power in an ultrasonic (QR500, Eco-sonic, Brazil) with titanium tip (0.5 cm inside solution). The solvents, same as from section 2.2.1, were applied separately. Residual solvent was removed, the extracts stored and the yield determined according to section 2.2.1.

2.2.4. Supercritical fluid extraction (SFE)

The supercritical fluid extractions were conducted according to Michielin et al. (2005), in a high-pressure dynamic unit described by Zetzl et al. (2003). The fixed bed of particles was formed with 15 g of samples (beetroot leaves or stems) inside the extraction column. The experimental conditions used were 250 bar, 40 °C and solvent flow rate of 0.5 kg $CO_2 h^{-1}$, during 4 h of extraction. The cosolvent assays from dried leaves were performed with ethanol and with the mixture ethanol-water (90:10), at concentrations of 4.0, 7.0 and 10% (w/w), related to the CO_2 mass. The SFE assays were conducted at least in duplicate. The extracts were collected in amber flasks and weighed (AY220, Shimadzu, São Paulo, Brazil). Residual solvent was removed, the extracts stored and the yield determined according to section 2.2.1.

2.3. Determination of total phenolic content (TPC)

Folin-Ciocalteu method (Kosar et al., 2005; Singleton et al., 1999) provided TPC (total phenolic content) values for the extracts from beetroot leaves and stems. Reaction mixture formed by 0.5 mL Folin-Ciocalteu reagent, 0.1 mL extract solutions (1667 mgL^{-1}), 7.9 mL distilled water, and 1.5 mL of 20% sodium carbonate solution. After 2 h rest, the absorbance at 765 nm was measured. Mean value of triplicate analysis was expressed as mg *Gallic acid* equivalent per gram of extract (mg GAE g^{-1}). Results were compared with non-processed raw materials (beetroot dried leaves and stems), prepared according to the international standard for green and black tea (ISO 14502-1, 2005). The TPC values from the extracts were compared to BHT performance determined by Cruz et al. (2017).

2.4. Antioxidant activity (AA)

2.4.1. Free radical scavenging potential by DPPH assay

Extracts from *Beta vulgaris* L. leaves and stems were evaluated by DPPH (1,1-diphenyl-2-picrylhydrazyl) method according to Mensor et al. (2001). Each extract was mixed with 0.3 mM DPPH ethanol solution to final concentrations of 500 μg/mL or 700 μg/mL, for leaves and stems, respectively. Antioxidant activity (AA%) was obtained from absorbance measured at 517 nm. The results were expressed as EC_{50}, i.e., the effective concentration to provide a 50% decrease in absorbance, calculated by a linear regression from AA% curves, and expressed as μg of extract/mL. The results considered the average of triplicate data. The DPPH values from the extracts were compared to result from BHT standard, according to Cruz et al. (2017).

2.4.2. ABTS•+ radical scavenging assay

This method follows the procedure by Re et al. (1999) with modifications. Synthetic vitamin E, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich Co, St Louis, USA) was used as a standard antioxidant. The ABTS [2,2-azino-bis-(3-ethylbenzotiazoline-6-sulfonic acid)] water solution (7.0 mMol) reacted with 140 mMol potassium persulfate and formed ABTS•+. The absorbance at 734 nm was measured 6 min from the initial mixing with 0.005 g extract samples. The results, compared to standard Trolox solutions, were expressed as μMTEAC/g (Trolox Equivalent Antioxidant Capacity per g extract), and present by average ± standard deviation of triplicate assays. The ABTS performance was also evaluated for the standard BHT, following the above-mentioned methodology replacing the extract sample with the BHT.

2.4.3. Ferric reducing antioxidant power (FRAP)

FRAP protocol was described by Benzie and Strain (1996), modified by Arnous et al. (2002). The reaction mixture was composed of 0.2 mL extract solutions and 0.2 mL Ferric Chloride (3 mM in 5 mM anhydrous citric acid). Blanks were prepared with 0.2 mL ethyl alcohol P.A. instead of extracts. The mixture was agitated and kept at 37 °C for 30 min. Then, 3.6 mL of TPTZ (2,4,6-tripyridyl-*s*-triazine, Sigma Aldrich) was added, vortexed and cooled for 10 min, for absorbance read at 620 nm. The extracts reducing power (P_R) was evaluated and compared to the standard curve of Trolox solutions (from 0 to 500 $\mu mol_{Trolox}/g_{extract}$). Results are presented by average ± standard deviation of triplicate

assays. The FRAP performance was also evaluated for the standard BHT, following the above-mentioned methodology replacing the extract sample with the BHT.

2.5. Profile of volatile compounds

The volatile fraction of the extracts was evaluated according to Aguiar et al. (2014) by gas chromatography coupled to mass spectrometry (GC 80 789A, MS 5975 MSD), using HP-5 mS 19091S-433 fused-silica capillary column (30 m × 0,250 mm) coated with 5% phenylpolymethylsiloxane (0.25 µm phase thickness) (Agilent Technologies). It consists of a headspace analysis, performed for extracts with high values of TPC and extraction yield. Column temperature started at 35 °C (2 min), raise to 80 °C (2 °C/min), 150 °C (4 °C/min), and 230 °C (8 °C/min), and kept for 2 min. Injector temperature, in splitless mode, was 250 °C; carrier gas was helium (1 mL/min); 1000 µL sample injected. Compounds identified comparing with spectral data library (NIST Willey 2.0 and 7.0).

2.6. Statistical analysis

A one-way analysis of variance (ANOVA) was applied to evaluate the results of global yield, TPC, and antioxidant potential. The significant differences detected at 95% confidence, with a p-value lower than 0.05, were evaluated by the Tukey test.

3. Results and discussion

3.1. Characterization of the raw material

Characterization of the raw materials (leaves and stem), presented in Table 1, was performed with dry samples, as used in the following procedures. Dried leaves presented carbohydrates and proteins as major constituents because it is a fibrous raw material with high nutritional value. The contents of carbohydrate, protein, fat, fiber and ash (Table 1), for leaves and stems, are comparable to literature data (Chhikara et al., 2019).

3.2. Global extraction yield (X_0)

The yield (X_0) values obtained by the various methods and solvents are presented in Table 2, for both raw materials, *Beta vulgaris* L. leaves and stems. From the leaves, the highest yield was 59.8 ± 0.8%, obtained by Soxhlet with water as the solvent. For the stems, the highest yield was reached by UAE (53.0 ± 0.7%), similar to Soxhlet (51 ± 2%), both methods performed with the mixture ethanol: water as solvent. In general, ethanol: water and water provided the best yields, probably due to the higher polarity, compared to CO_2 and hexane, and also to the solubility of different classes of components. For instance, high extraction yields obtained by water and by ethanol-water as solvents (Table 2), are also related to solubilization of carbohydrates and other substances present in *Beta vulgaris* leaves (Biondo et al., 2014).

Table 1
Nutritional composition of the beetroot leaves and stems.

Nutrient	Leaves (g/100 g)	Stems (g/100 g)
Total carbohydrates[a]	36.77	49.69
Protein[b]	25.13	13.66
Fat[c]	4.22	1.84
Crude fiber[c]	6.72	10.09
Moisture and volatile matter[c]	8.42	6.62
Ashes[c]	18.78	18.10

[a] RDC n° 360.
[b] AOAC n° 991.20.
[c] IAL, 2008.

For the stems, higher yields were provided by hexane and ethanol: water. The yield results from beetroot residues indicate the presence of mainly polar compounds in these raw materials. This is a very promising behavior since antioxidant components are mostly polar substances, such as phenolic acids, flavonoids, and betalain.

High yield performance from Soxhlet method is attributed to the higher temperatures applied, compared to other procedures, reducing surface tension and viscosity, and favoring the solubilization of different components. At these temperatures, the solvent reaches the active sites within the matrix, allowing solute-matrix interactions and increasing solute volatility (Markom et al., 2007). Cruz et al. (2017) observe the same behavior for extraction from *Butia catarinensis* seeds. Ultrasound-assisted extractions (UAE) provided yields similar to Soxhlet, with the highest value for the stems by water: ethanol (53.0 ± 0.7%). The high water: ethanol efficiency for the stem may be due to the high content of carbohydrates (49.69 g/100 g) in these samples. UAE is based on high-frequency ultrasonic waves that produce cavitation. The cavitation induces cell wall rupture of the solid matrix, favoring the solvent penetration and mass transfer, increasing extraction yield (Toma et al., 2001).

SFE with 10% water: ethanol (90:10) as cosolvent increased the yield to 14.45 ± 1.3% for the leaves and to 6 ± 1% for the stems. This is caused by the increase in polar compounds solubilization with enhancing cosolvent concentration due to an increase in solvent polarity. Therefore, different performances can be obtained by manipulating the modifier concentration, because solute–cosolvent interactions also contribute to cosolvent effects (Casas et al., 2007).

The variables with high influence in the extraction methods were the solvent polarity and temperature. Higher yields were provided by more polar solvents, even for SFE, which the yield increased with the use of polar cosolvent. As for the temperature, Soxhlet extraction, performed at solvent boiling temperature, provided high extraction yields.

3.3. Total phenolic content (TPC)

The results of total phenolic content (TPC) for all extract samples are represented in Table 3 and compared to the standard synthetic antioxidant BHT. The data from the recovered extracts can be compared to the ones obtained from the *in natura* dried samples, determined by standard procedure for total phenolic compounds.

TPC results from leaves varied from 1.92 to 99 $mg_{GAE}/g_{extract}$ and from stems from 4.26 to 98 $mg_{GAE}/g_{extract}$. Among the several extraction protocols and solvents, SFE with cosolvent showed the best TPC results for both raw materials, reaching highest values with ethanol: water (90:10) as a cosolvent, i.e., 99 ± 4 and 98 ± 6 $mg_{GAE}/g_{extract}$ for leaves and stems, respectively. The UAE with ethanol for the leaves also provided high TPC result (73 ± 4 $mg_{GAE}/g_{extract}$), followed by Soxhlet with ethanol and water: ethanol (45 ± 3 and 47 ± 2 $mg_{GAE}/g_{extract}$). Similarly, for the stems, UAE with ethanol provided TPC of 33 ± 4 $mg_{GAE}/g_{extract}$, while SOX with ethanol and ethanol: water reached 55.0 ± 0.7 and 54 ± 3 $mg_{GAE}/g_{extract}$, respectively.

SFE is, depending on the process parameters, a highly selective method, due to easy control of the solvent characteristics (REVERCHON, DE MARCO, 2006). Although SFE provided yield values lower than Soxhlet, the high-pressure method seems to be selective towards some phenolic compounds as presented in Table 3, while Soxhlet method, possibly due to the high process temperature, recovers less phenolic compounds, especially as they are thermosensitive compounds. Compared to the dried samples (1.92 and 4.26 mg $_{GAE}/g_{extract}$, for leaves and stems respectively), it is observed that all extraction methods (SOX, MAC, UAE and SFE) enhanced the recovery of phenolic substances (Sartori et al., 2013), especially SFE with CO_2 + 7% EtOH: H_2O (90/10) for leaves and CO_2 + 10% EtOH for stems.

Comparing the values of extraction yield with the TPC data it is

Table 2
Global yield (X_0) of *Beta vulgaris* L. leaves and stem extracts for low-pressure extraction and supercritical fluid extraction.

Extraction procedure[1]	Solvent[2]	Leaves	Stems	Leaves	Stems
		Dry weigth (g)		X_0 (%, w/w)[3]	
SOX	H_2O	2.99	2.36	$59.8^{a,A} \pm 0.8$	$47.2^{b} \pm 0.7$
	H_2O:EtOH	1.87	2.55	$37.4^{b,B} \pm 0.7$	$51^{ab} \pm 2$
	EtOH	1.05	0.45	$21^{d,D} \pm 1$	$9.0^{f} \pm 0.7$
	Hx	0.09	0.35	$1.8^{ij,GH} \pm 0.7$	$7.1^{fg} \pm 0.6$
MAC	H_2O	0.34	0.79	$6.7^{fg,EF} \pm 1.5$	$15.8^{e} \pm 0.3$
	H_2O:EtOH	0.26	1.45	$5.3^{fgh,EF} \pm 0.5$	$29^{d} \pm 1$
	EtOH	0.08	0.25	$1.6^{ij,GH} \pm 0.7$	$5.0^{g} \pm 0.4$
	Hx	0.38	1.75	$7.6^{f,E} \pm 0.7$	$35^{c} \pm 2$
UAE	H_2O	1.56	1.84	$31.3^{c,C} \pm 06$	$36.7^{c} \pm 0.5$
	H_2O:EtOH	1.95	2.65	$38.9^{b,B} \pm 0.1$	$53.0^{a} \pm 0.7$
	EtOH	0.20	0.24	$4.04^{ghi,FG} \pm 0.2$	$4.7^{g} \pm 0.2$
	Hx	0.01	0.03	$0.15^{j,H} \pm 0$	$0.6^{h} \pm 0.1$
SFE (250 bar/40 °C)	CO_2 + 4% EtOH	0.36	ND	$2.4^{hij,\beta} \pm 0.8$	ND
	CO_2 + 4% EtOH:H_2O	0.16	ND	$1.1^{ij,\beta} \pm 0.2$	ND
	CO_2 + 7% EtOH	0.25	ND	$1.17^{ij,\beta} \pm 0.01$	ND
	CO_2 + 7% EtOH:H_2O	0.31	ND	$2.05^{ij,\beta} \pm 0.41$	ND
	CO_2 + 10% EtOH	0.61	0.07	$4.06^{ghi,\beta} \pm 0.08$	$0.5^{h} \pm 0.3$
	CO_2 + 10% EtOH:H_2O	2.17	0.9	$14.45^{e,\alpha} \pm 1.3$	$6^{fg} \pm 1$

[1]SOX: Soxhlet extraction; MAC: maceration; UAE: ultrasound-assisted extraction; SFE: supercritical fluid extraction.
[2]H_2O: water; EtOH: ethanol; Hx: hexane; CO_2: carbon dioxide; H_2O:EtOH mixture with 90% ethanol in water.
[3]Equal letters in the same column indicate no significant difference ($p < 0.05$), as follows: (a) Lower-case Latin letters compare all results; (b) Greek letters compare SFE results (c) Upper-case Latin letters compare results of SOX, MAC and UAE; ND: not determined.

Table 3
Total phenolic content (TPC) for *Beta vulgaris* L. extracts from leaves and stems.

Extraction method[1]	Solvent[2]	TPC (mg GAE/$g_{extract}$)[3]	
		Leaves	Stems
	Dry samples: Metanol 70%*	$1.92^{g,E}$	4.26^{i}
SOX	H_2O	$3^{g,E} \pm 2$	$11^{i} \pm 5$
	H_2O:EtOH	$45^{d,C} \pm 3$	$54^{c} \pm 3$
	EtOH	$47^{d,C} \pm 2$	$55.0^{c} \pm 0.7$
	Hx	$16^{ef,D} \pm 2$	$24^{defg} \pm 4$
MAC	H_2O	$17^{ef,D} \pm 3$	$16^{fghi} \pm 1$
	H_2O:EtOH	$19^{ef,D} \pm 0$	$15^{ghi} \pm 2$
	EtOH	$21.3^{e,D} \pm 0.6$	$22^{efghi} \pm 2$
	Hx	$21^{e,D} \pm 2$	$31^{de} \pm 5$
UAE	H_2O	$21^{e,D} \pm 2$	$14^{hi} \pm 1$
	H_2O:EtOH	$23^{e,D} \pm 2$	$25^{def} \pm 5$
	EtOH	$73^{c,B} \pm 4$	$33^{d} \pm 4$
	Hx	$22^{e,D} \pm 4$	$14^{ghi} \pm 4$
SFE (250 bar/40 °C)	CO_2 + 4% EtOH	$6.2^{g,\zeta} \pm 0.6$	
	CO_2 + 4% EtOH: H_2O	$70^{c,\gamma} \pm 4$	
	CO_2 + 7% EtOH	$49^{d,\delta} \pm 6$	
	CO_2 + 7% EtOH: H_2O	$99^{b,\beta} \pm 4$	
	CO_2 + 10% EtOH	$18^{ef,\varepsilon} \pm 3$	$98^{b} \pm 6$
	CO_2 + 10% EtOH:H_2O	$11^{fg,\varepsilon\zeta} \pm 3$	$23^{defgh} \pm 2$
Standard BHT		$266.4^{A,\alpha,a} \pm 0.4$	$266.4^{a} \pm 0.4$

[1]SOX: Soxhlet extraction; MAC: maceration; UAE: ultrasound-assisted extraction; SFE: supercritical fluid extraction.
[2]H_2O: water; EtOH: ethanol; Hx: hexane; CO_2: carbon dioxide; H_2O: EtOH mixture with 90% ethanol in water.
[3]Equal letters in the same column indicate no significant difference ($p < 0.05$), as follows: (a) Lower-case Latin letters compare all results; (b) Greek letters compare SFE results (c) Upper-case Latin letters compare results of SOX, MAC and UAE.
ND: not determined.
* Procedure ISO 14502-1, 2005.

possible to detect the high selectivity of the SFE procedure. For instance, for beetroot leaves, although SFE with cosolvent produced low extraction yield compared to other methods, especially UAE (2.05 ± 0.41% for SFE with 7% ethanol:water mixture and 38.9 ± 0.1% for UAE with ethanol:water mixture), the TPC results were higher, i.e., the same procedures provide TPC of 99 ± 4 and 23 ± 2 mg GAE/$g_{extract}$, respectively. This behavior was found for most SFE data compared to low pressure extraction methods. This shows the high selectivity of the supercritical mixture towards phenolic substances and that the high yield of the other extraction methods is not directly proportional to a high TPC. The same behavior was detected for the beetroot stems, as presented in Tables 2 and 3

Additionally, the results of extraction yield and TPC were correlated using Pearson analysis to investigate the selectivity of the extraction procedures for beetroot residues. From that, extraction yield (X_0) shown a higher linear correlation with TPC for SFE for leaves (−0.50), while the low pressure methods (SOX, MAC and UAE) provided a less strong correlation (−0.18). These results confirm the better SFE selectivity towards phenolic substances from beetroot residues, compared to SOX, MAC and UAE methods.

The TPC obtained from this work from beetroot leaves and stems were superior than that obtained by Guiné et al. (2018) for the vegetal root using the mixture ethanol:water (50:50 v/v). Also, several literature studies show that SFE with ethanol: water as cosolvent increases recovery of phenolic acids due to solvent polarity enhancement (Pekic et al., 1998; Yilmaz, Toledo, 2006; Biscaia and Ferreira, 2009). Phenolic compounds are excellent oxygen radical scavengers and therefore are potentially associated with antioxidants. Also, the antioxidant capacity is specially provided by phenolic compounds such as flavones, isoflavones, flavonoids, anthocyanins, coumarin lignans, catechins and isocatechins (Aqil et al., 2006; Giada, 2013).

3.4. Antioxidant activity

Because the antioxidant activity (AA) is due to multiple reactions and mechanisms, a single method does not reflect accurately all the antioxidant activity from a complex system. Therefore, three antioxidant assays were conducted to clarify different aspects of the antioxidant capacity from extracts from beetroot residues. The DPPH, ABTS and FRAP methods are simple and fast assays to detect the antioxidant

Table 4
Antioxidant activities (AA) determined by DPPH, ABTS and FRAP methods, for the extracts from *Beta vulgaris* L. leaves and stems.

Extraction method[1]	Solvent[2]	Leaves			Stems		
		DPPH	ABTS	FRAP	DPPH	ABTS	FRAP
		EC_{50} (μG/mL)	TEAC ($\mu mol_{Trolox}/g_{extract}$)	P_R ($\mu mol_{Trolox}/g_{extract}$)	EC_{50} (μG/mL)	TEAC ($\mu mol_{Trolox}/g_{extract}$)	P_R ($\mu mol_{Trolox}/g_{extract}$)
SOX	H_2O	$1085^{bc,BC} \pm 43$	$4.4^{g,H} \pm 0.1$	$134^{g,EF} \pm 4$	$1294.6^{b} \pm 0.8$	$49.20^{ef} \pm 0.03$	$5.5^{g} \pm 0.8$
	H_2O:EtOH	$523^{ef,EF} \pm 19$	$58^{fg,FGH} \pm 12$	$91^{h,G} \pm 6$	$1232^{bc} \pm 186$	$6.70^{h} \pm 0.05$	$17^{fg} \pm 3$
	EtOH	$302^{gh,GH} \pm 7$	$586^{a,A} \pm 82$	$182^{d,C} \pm 12$	$59.4^{f} \pm 3.2$	$730^{a} \pm 14$	$63^{def} \pm 3$
	Hx	$398^{fg,FG} \pm 9$	$14.9^{g,GH} \pm 0.2$	$123^{g,F} \pm 10$	$800^{cde} \pm 5$	$24.6^{fgh} \pm 0.2$	$156^{c} \pm 0$
MAC	H_2O	$673^{de,DE} \pm 25$	$142^{de,DEF} \pm 14$	$9^{k,I} \pm 4$	$797^{cde} \pm 2$	$10.30^{h} \pm 0.01$	$6.4^{g} \pm 0.0$
	H_2O:EtOH	$304^{gh,GH} \pm 19$	$97^{def,DEFG} \pm 2$	$18^{k,I} \pm 5$	$702^{de} \pm 40$	$41.6^{fg} \pm 0.4$	$7^{g} \pm 4$
	EtOH	$139^{hi,GH} \pm 7$	$254^{c,C} \pm 24$	$259^{a,A} \pm 5$	$518^{ef} \pm 4$	$11^{h} \pm 3$	$163^{c} \pm 11$
	Hx	$872^{cd,CD} \pm 2$	$149^{de,DE} \pm 24$	$146^{ef,DE} \pm 12$	$1131^{bcd} \pm 35$	$0^{h} \pm 0$	$30^{efg} \pm 2$
UAE	H_2O	$< 1700^{a,A}$	$384^{b,B} \pm 27$	$6^{k,I} \pm 1$	$< 2000^{a}$	$9^{h} \pm 4$	$0^{g} \pm 0$
	H_2O:EtOH	$732^{de,DE} \pm 1$	$82^{efg,EFGH} \pm 1$	$45.1^{j,H} \pm 0.7$	$< 1500^{b}$	$17^{gh} \pm 3$	$1.5^{g} \pm 0$
	EtOH	$275^{ghi,GH} \pm 4$	$384^{b,B} \pm 6$	$140^{fg,DEF} \pm 3$	$1147^{bcd} \pm 60$	$83^{d} \pm 2$	$30^{efg} \pm 2$
	Hx	$1231^{b,B} \pm 22$	$173^{d,CD} \pm 42$	$161^{e,CD} \pm 3$	$1428^{b} \pm 69$	$41.4^{fg} \pm 0.5$	$87^{d} \pm 5$
SFE (250 bar/40 °C)	CO_2 + 4% EtOH	$334^{gh,\alpha} \pm 5$	$111^{def,\gamma\delta} \pm 13$	$130.2^{g,\delta} \pm 0.7$	–	–	–
	CO_2 + 4% EtOH:H_2O	$266^{ghi,\beta} \pm 31$	$142^{de,\beta\gamma} \pm 12$	$124^{g,\delta} \pm 0$	–	–	–
	CO_2 + 7% EtOH	$346^{gh,\alpha} \pm 6$	$113^{def,\gamma\delta} \pm 14$	$187^{d,\gamma} \pm 2$	–	–	–
	CO_2 + 7% EtOH:H_2O	$322^{gh,\alpha} \pm 18$	$139^{de,\beta\gamma\delta} \pm 19$	$218^{c,\beta} \pm 6$	–	–	–
	CO_2 + 10% EtOH	$326^{gh,\alpha} \pm 11$	$99^{def,\delta} \pm 24$	$240^{ab,\alpha} \pm 3$	$< 1500^{b}$	$137^{c} \pm 33$	$633^{a} \pm 54$
	CO_2 + 10% EtOH:H_2O	$150^{hi,\gamma} \pm 7$	$157^{de,\beta} \pm 2$	$71^{i,e} \pm 4$	$738^{cde} \pm 33$	$79^{de} \pm 12$	$70^{de} \pm 4$
BHT*		$67^{H,\delta,i}$	$391.9^{B,\alpha,b} \pm 0.6$	$215^{bc,\beta,B} \pm 2$	67^{f}	$391.9^{b} \pm 0.6$	$215^{b} \pm 2$

[1]SOX: Soxhlet extraction; MAC: maceration; UAE: ultrasound-assisted extraction; SFE: supercritical fluid extraction.
[2]H_2O: water; EtOH: ethanol; Hx: hexane; CO_2: carbon dioxide; H_2O: EtOH mixture with 90% ethanol in water.
[3]Equal letters in the same column indicate no significant difference ($p < 0.05$), as follows: (a) Lower-case Latin letters compare all results; (b) Greek letters compare SFE results (c) Upper-case Latin letters compare results of SOX, MAC and UAE.
[4] Total phenolic content. [4] EC_{50}: Effective concentration at 50% AA referent to DPPH method. TEAC: Trolox-equivalent antioxidant activity. P_R: Reducing power. *BHT: standard. ND: not determined.

capacity from fruits and vegetables (Antolovich et al., 2001). The AA results for all beetroot residue extracts are presented in Table 4 and compared to the values for the standard BHT.

The DPPH data are expressed as EC_{50}, which represent the effective extract concentration to achieve 50% antioxidant potential. From leaves samples, ethanol provided better DPPH values (lower EC_{50}) for SOX, MAC and UAE. Otherwise, SFE provided good DPPH performance using ethanol and ethanol:water mixtures as cosolvents (EC_{50} values from 150 ± 7 to 346 ± 6 μg/mL), probably because of the selectivity extraction of low molecular weight phenolic compounds, which contribute more to antioxidant potential than the carbohydrates fractions recovered by other extraction methods. The use of ethanol: water as a cosolvent in SFE benefits the antioxidant potential of the extract. According to Campos et al. (2008), EC_{50} values below 250 μg/mL represent good antioxidant agent, and this value was detected for MAC with EtOH and for SFE with 10% EtOH: H_2O, with the results of 139 ± 7 and 150 ± 7 μg/mL, respectively. The EC_{50} data from the leaves were better than found for several natural materials such as *Butia catarinensis* (Cruz et al., 2017) and Merlot grape (*Vitis vinifera*) pomace (Mezzomo et al., 2013) indicating beetroot leaves as a viable source of antioxidant compounds. Otherwise, beetroot stems were not as versatile as the leaves, because only the extract recovered by SOX with EtOH provided good DPPH performance (EC_{50} of 59.4 ± 3.2 μg/mL), statistically equal ($p < 0.05$) to the BHT result.

The ABTS analysis is an excellent tool to determine the antioxidant potential of hydrogen donor compounds and chain terminator compounds and is recommended to measure the activity of hydrophilic and lipophilic compounds (Re et al., 1999). The highest ABTS results were obtained by Soxhlet with ethanol, reaching 586 ± 82 $\mu M_{TEAC}/g_{extract}$ for beetroot leaves, while for stems was up to 730 ± 14 $\mu M_{TEAC}/g_{extract}$. Soxhlet with ethanol provided the highest results for both by-products, differing significantly from others results, and also higher than BHT performance (214.9 ± 1.77 $\mu M_{TEAC}/g_{extract}$. Water and hexane as solvents also provided good ABTS results. This behavior agrees with ABTS characteristic to detect activity from hydrophilic and lipophilic compounds, recovered by a wide range of solvents. Compared to the control (BHT), the best ABTS values were obtained mostly by ethanolic extracts, particularly for the beetroot leaves, showing the good antioxidant potential of this sample. Bergamaschi (2010) obtained ABTS results for beetroot stem extracts recovered by UAE. The values were 121.48 $\mu M_{TEAC}/g_{extract}$ (with ethanol: water) and 79.67 $\mu M_{TEAC}/g_{extract}$ (with water), similar to results from the present work (83 $\mu M_{TEAC}/g_{extract}$) UAE with ethanol for beetroot stems.

FRAP results from leaves were up to 259 ± 5 $\mu M_{TEAC}/g_{extract}$ for MAC with ethanol, a result statistically similar to SFE with 10% ethanol (240 ± 3 $\mu M_{TEAC}/g_{extract}$). The same SFE procedure also provided the best value for the stems (633 ± 33 $\mu M_{TEAC}/g_{extract}$). These results express the importance of ethanol and CO_2 as solvents for the antioxidant fraction detected by FRAP method. Halvorsen et al. (2009) found FRAP values for the beetroot of 1980 μmol/g, a value much superior to the present work. Although, it is important to observe the difference in the raw materials, i.e., the authors used the mashed vegetable, while in the present work the dry leaves and stems were studied. Comparing the results from the various extracts with the BHT value (215 ± 2 $\mu M_{TEAC}/g_{extract}$) we observe a very good performance of various extraction methods, particularly SFE for the beetroot leaves and stems. This behavior could be justified by the high selectivity of the supercritical extraction procedure toward active components such as low molecular

weight phenolic substances, as presented by the results from TPC.Also, Shaidi and Naczk (1995) point a wide range of antioxidant compounds present in foodstuff, from simple molecules to highly polarized ones, representing a large variety of components (phenolic acids, anthocyanins, tannins and others.

Results of extraction yield (X_0) and antioxidant activity were correlated by Pearson analysis for the beetroot leaves. From that, extraction yield (X_0) shown a higher linear correlation with DPPH for SFE for leaves (−0.88), while the low pressure methods (SOX, MAC and UAE) provided a less strong correlation (0.23). The same behavior was detected comparing yield with ABTS and FRAP assays. For instance, SFE provided higher linear Pearson correlations (0.54 and −0.59 for ABTS and FRAP, respectively) compared to other methods (0.19 and −0.24, respectively).

In general, comparing the yield values (Table 2) with the antioxidant performance (Table 4), it is possible to detect that the FRAP results from the samples by SFE were significantly higher compared to other extraction methods. The same behavior was observed by ABTS values. These results suggest a better SFE selectivity towards compounds detected by FRAP and ABTS, compared to other extraction methods.

Also, comparing FRAP and by ABTS data with the ones obtained by the standard BHT, the good performance of the SFE is clear. These results suggest that beetroot leaves and stems are a good source of natural antioxidants, properly recovered by SFE, and useful for further applications such as in food or cosmetic products (Neelwarne, 2013; Dlim et al., 2013).

3.5. Gas chromatographic profile

The chemical profile of recovered extracts was detected by GC-MS in order to provide an overview of the volatile compounds of interest. The results from leaves and stems are presented in Table 5. The chromatographic composition was evaluated for selected extracts based on the results of yield, TPC and ABTS. Considering this, the samples analyzed were: from the leaves (SOX-ethanol; UAE-ethanol; SFE- 7% ethanol: water); and from the stems (SOX-ethanol; SFE-10% ethanol).

The samples showed a variety of volatile compounds, including alcohols, alkanes, aldehydes and ketones. Comparing the results from the leaves and stems samples, different compounds were detected as main components identified by GC-MS, particularly for the alcohol fraction. For instance, for the stem sample SFE-10% ethanol, the 1-pentanol was the representative compound, while for SOX and UAE samples, for leaves and stems, the isooctanol (commercial name 2-ethyl-1-hexanol) was the main substance detected, which is an important compound used as a fragrance ingredient (Mc Ginty et al., 2010). Also, SOX-ethanol samples showed the largest variety of compounds, for leaves and stems. This can be attributed to the lack of selectivity presented by Soxhlet method, providing higher extraction yield due to high process temperature and extraction time. Therefore, the main identified components from all samples present important characteristics, useful for cosmetic, food and pharmaceutic products, and an adequate definition of the extraction method must combine yield with product quality. For instance, SFE with 10% ethanol:water from the stem was able not only to recover an extract rich in phenolics compounds (Table 3), but also provided the highest relative area of volatile for 1-pentanol (Table 5). These results contribute to a biorefinery concept with the simultaneous recovery of different products from a complex raw material such as the aerial parts of beetroot.

Table 5
Gas chromatographic relative peak area (%) of extracts from *Beta vulgaris* L. leaves and stem obtained by low-pressure extraction (LPE) and by supercritical fluid extraction (SFE).

Identified component	t_r(min)	Extraction methods*				
		1	2	3	4	5
1-Pentanol	4.96	–	–	–	–	23.62
1-butanol -2-methyl	5.71	–	–	–	–	0.97
Dimethylsilanediol	6.27	0.50	–	–	–	–
2-Heptanone	11.64	–	–	–	–	0.21
2-Ethyl-1-Hexanol	12.28	5.36	28.45	1.32	18.01	1.22
3- Octanone	17.85	–	–	–	–	–
1,2,4-trimethyl-benzene	17.95	3.70	–	–	–	–
1,2,3-trimethyl-Benzene	19.89	1.43	–	–	–	–
Undecane	21.78	2.24	–	–	2.20	–
Hexadecane	45.37	–	1.09	–	–	–
2-methyl-decahydronaphthalene or Tetramethyl-Pyrazine	24.67	2.99	–	–	4.65	–
1-Methyl-decahydronaphthalene	26.97	0.87	–	–	1.32	–
2-methyl-undecane	28.70	1.38	–	–	–	–
2,6-dimethyl-decahydro-naphthalene	28.89	1.34	–	–	–	–
3-methyl-undecane	29.08	1.78	–	–	–	–
2-methyl-undecane	29.68	–	–	–	1.21	–
2,3,5-trimethyl-Decane	31.50	–	–	–	8.79	–
4,6-dimethyl-undecane	30.88	7.31	–	–	–	–
(E) -5-Dodecene or 3,6-dimethyl-undecane	31.09	2.80	1.00	–	3.99	–
Heptadecane or Dodecane	31.52	7.90	7.01	–	–	–
3,6-dimethyl-undecane	31.62	3.09	–	–	–	–
2-buthyl-1,1,3-trimethyl-ciclohexane	32.25	–	–	–	0.85	–
4-methylpenthyl-cyclohexane	33.09	–	–	–	0.91	–
Cyclotetradecane	33.29	–	–	–	0.56	–
2,10-dimethyl-undecane	34.33	–	–	–	0.47	–
3-methylnonane or 2,6-dimethyloctane	34.71	0.42	–	–	0.78	–
Tetradecane	39.43	–	4.76	–	–	–
4- (2,6,6-Trimethylcyclohex-1,3-dienyl) but-3-em-2-one	42.19	–	–	–	–	–
[1R- (1R*,4Z,9S*)]-4,11,11-Trimethyl-8-methylene-Bicyclo[7.2.0] undec-4-ene	44.53	–	–	–	–	0.15
2,6-Bis (1,1-dimethylethyl) -4- (1-oxopropyl) phenol	46.22	0.25	–	–	–	–
4-octadecyl-morphine	50.34	0.38	–	–	–	–
2,2-dimethoxy-1,2-diphenyl-ethanone	50.46	0.79	–	–	–	–

*__Samples from the extract methods:__ 1: Soxhlet-ethanol from leaves; 2: Ultrasound-ethanol from leaves; 3: SFE- 7% ethanol + water (90:10) from leaves; 4: Soxhlet-ethanol from stem; 5: SFE-10% ethanol from stem.

4. Conclusions

The use of different extraction methods to recover bioactive compounds from beetroot residues suggest that, besides the well-known nutritional aspects, the aerial parts from *Beta vulgaris* L. are also valuable sources of antioxidants. The comparison between conventional extraction methods and high-pressure technology showed good results for the SFE, especially in respect to TPC and antioxidant potential, by ABTS and FRAP methods. The high AA from most supercritical extracts, allied with the low yield, confirm the high selectivity of the supercritical CO_2 towards phenolic compounds, performance corroborated by data correlation (Pearson method), comparing SFE with low pressure extractions. Ethanol also play a significant role in all extraction methods (SOX, MAC, UAE and SFE) in order to provide the best combination of yield, TPC and antioxidant potential. Within the methods evaluated in this study, SFE with cosolvent (CO_2 + 7% EtOH:H_2O) provided the most efficient recovery of phenolic compounds from beetroot leaves, while for the stems the SFE with 10% ethanol resulted in the highest antioxidant behavior by FRAP method. Finally, these unconventional edible parts from beetroot, normally neglected from the industrial view, can be reused for the recover valuable extracts, for further applications such as in food or in the pharmaceutical industry.

Practical application

The practical application of the present research is related to the evaluation of a viable route to treat a common biomass from industrial processing of beetroot, mostly from sugar and ethanol production. As a result, the low value vegetable aerial parts, the leaves and the stems, can be useful as a raw material to obtain antioxidant compounds by means of green extraction methods.

Acknowledgments

The authors wish to thank CNPq (projects number 454025/2014 and 473153/2012-2) and CAPES, Brazilian funding agencies, for the financial support and scholarship which sponsored this work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101169.

References

Aguiar, M.C.S., Silvério, F.O., Pinho, G.P., Lopes, P.S.N., Fidêncio, P.H., Ventura, S.J., 2014. Volatile compounds from fruits of *Butia capitata* at different stages of maturity and storage. Food Res. Int. 62, 1095–1099.

Antolovich, M., Prenzler, P.D., Patsalides, E., Mcdonald, S., Robards, K., 2001. Methods for testing antioxidant activity. Analyst 127, 183–198.

Aqil, F., Ahmad, I., Mehmood, Z., 2006. Antioxidant and free scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol. 30, 177–183.

Arnous, A., Makris, D.P., Kefalas, P., 2002. Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. J. Food Anal. 15, 655–665. https://doi.org/10.1006/jfca.2002.1070.

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., SAHENA, F., 2013. Techniques for extraction of bioactive compounds from plant materials: a review. J. Food Eng. 117, 426–436.

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239, 70–76.

Bergamaschi, K.B., 2010. Antioxidant Capacity and Chemical Composition of Vegetable Wastes Aiming at Their Use. Dissertation (Master science and technology of food). University of São Paulo. Piracicaba.

Biondo, P.F., Boeing, J.S., Barizão, E.O., Souza, N.E., Matsushita, M., Oliveira, C.C., Boroski, M., Visentainer, J.V., 2014. Evaluation of beetroot (Beta vulgaris L.) leaves during its developmental stages: a chemical composition study. Food Sci. Tecnol. (Campinas) 34 (1), 94–101. Jan.-Mar. https://doi.org/10.1590/S0101-20612014005000007.

Biscaia, D., Ferreira, S.R.S., 2009. Propolis extracts obtained by low-pressure methods and supercritical fluid extraction. J. Supercrit. Fluids 51, 17–23.

Campos, L.M.A.S., Leimann, F.V., Pedrosa, R.C., Ferreira, S.R.S., 2008. Free radical scavenging of grape pomace extracts from Cabernet sauvingnon (*Vitis vinifera*). Bioresour. Technol. 99, 8413–8420.

Casas, L., Matell, C., Rodríguez, M., Torres, A., Macías, F.A., Martínez De La Ossa, E., 2007. Effect of the addition of cosolvent on the supercritical fluid extraction of bioactive compounds from *Helianthus annuus* L. J. Supercrit. Fluids 41, 43–49.

Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., Panghal, A., 2019. Bioactive compounds of beetroot and utilization in food processing industry: a critical review. Food Chem. 272, 192–200.

Cruz, P.N., Pereira, T.C.S., Guindani, C., Oliveira, D.A., Rossi, M.J., Ferreira, S.R.S., 2017. Antioxidant and antibacterial potential of butia (*Butia catarinensis*) seed extracts obtained by supercritical fluid extraction. J. Supercrit. Fluids 119, 229–237.

Dlim, M.M., Alsabri, S.G., Mohamed, S.S., Zetrini, A.E., Salem, A.A.H., Auzi, A.A., Mohamed, S.B., 2013. Use of Beta Vulgaris as natural coloring agent for foods and cosmetics in Libya. J. Chem. Pharm. Res. 11, 340–345.

Duraisam, R., Salelgn, K., Berekete, A.K., 2017. Int. J. Eng. Trends Technol. 43 (4) ISSN: 2231-5381.

Giada, M.L.R., 2013. Food phenolic compounds: main classes, sources and their antioxidant power. Intech 87–112. https://doi.org/10.5772/51687.

Guiné, R.P.F., Gonçalves, F., Lerat, C., Idrissi, T.E., Rodrigi, E., Correia, P.M.R., Gonçalves, J.C., 2018. Extraction of phenolic compounds with antioxidant activity from beetroot (Beta vulgaris L.). Curr. Nutr. Food Sci. 14 (4), 350–357.

Halvorsen, B.L., Holte, K., Myhrstad, M.C.W., Barikmo, I., Hvattum, E., Remberg, S.F., Wold, A.N., Haffner, K., Baugerod, H., Andersen, L.F., Moskkaug, J., Jacobs, D.V.R., Blomhoff, R., 2009. A systematic screening of total antioxidants in dietary plants. J. Nutr. 132, 461–471.

Herrero, M., Cifuentes, A., Ibañez, E., 2006. Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae. A review. Food Chem. 98, 136–148.

IAL - Instituto Adolfo Lutz (São Paulo), 2008. In: In: Zenebon, O., Pascuet, N.S., Tiglea, P. (Eds.), Métodos físico-químicos para análise de alimentos, vol. 4. pp. 117.

ISO 14502-1, 2005. International Standard. Determination of Substances Characteristic of Green and Black Tea. Available at: https://www.iso.org/obp/ui/#iso:std:iso:14502:-2:ed-1:v1:en.

Kosar, M., Dorman, H.J.D., Hiltunen, R., 2005. Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem. 91, 525–533.

Krajnc, D., Mele, M., Glavič, P., 2007. Improving the economic and environmental performances of the beet sugar industry in Slovenia: increasing fuel efficiency and using by-products for ethanol. J. Clean. Prod. 15, 1240–1252.

Markom, M., Hasan, M., Daud, W.R.W., Singh, H., Jahim, J., 2007. Extraction of hydrolysable tannins from *Phyllanthus niruri* Linn.: effects of solvents and extraction methods. Separ. Purif. Technol. 52, 487–496.

Mc Ginty, D., Scognamiglio, J., Letizia, C.S., Api, A.M., 2010. Fragrance material review on 2-ethyl-1-hexanol. Food Chem. Toxicol. 48 (S4), S115–S129.

Mensor, L.L., Menezes, F.S., Leitão, G.G., Reis, A.S., Santos, T.C., Coube, C.S., Leitão, S., 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 15, 127–130.

Mezzomo, N., Oliveira, D.A., Ferreira, S.R.S., 2013. Antioxidant potential of extracts from processing residues from Brazilian food industries. Food Nutr. Sci. 4, 211–218.

Michielin, E.M.Z., Bresciani, L.F.V., Danielski, L., Yunes, R.A., Ferreira, S.R.S., 2005. Composition profile of horsetail (Equisetum giganteum L.) Oleoresin: comparing SFE and organic solvent extraction. J. Supercrit. Fluids 33, 131–138.

Michielin, E.M.Z., Salvador, A.A., Riehl, C.A.S., Smânia, A., Smânia, E.F.A., Ferreira, S.R.S., 2009. Chemical composition and antibacterial activity of Cordia verbenacea extracts obtained by different methods. Bioresour. Technol. 100, 6615–6623.

Mikolajczyk-Bator, K., Blaszczyk, A., Czyzniejewski, M., Kachlicki, P., 2016. Characterisation and identification of triterpene saponins in the roots of red beets (*Beta vulgaris L.*) Using two HPLC-MS systems. Food Chem. 192, 979–990.

Neelwarne, B., 2013. Red Beet Biotechnology: Food and Pharmaceutical Applications. Springer Science Business Media, New York Cap. 14 and 16.

Ninfali, P., Angelino, D., 2013. Nutritional and functional potential of *Beta vulgaris cicla* and *rubra*. Fitoterapia 89, 188–199.

Oliveira, D.A., Angonese, M., Gomes, C., Ferreira, S.R.S., 2016. Valorization of passion fruit (Passiflora edulis sp.) By-products: sustainable recovery and biological activities. J. Supercrit. Fluids 111, 55–62.

Pekič, B., Kovač, V., Alonso, E., Revilla, E., 1998. Study of the extraction of proanthocyanidins from grape seeds. Food Chem. 61 (1/2), 201–206.

Rana, M.K., 2016. Salad Crops: Dietary Importance. Reference Module in Food Science. Encyclopedia of Food and Health, pp. 665–672. https://doi.org/10.1016/B978-0-12-384947-2.00602-4.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biol. Med. 26, 1231–1237.

Reichardt, C., 2003. Solvents and Solvent Effects in Organic Chemistry, third ed. Wiley-VCH Publishers.

Reverchon, E., De Marco, I., 2006. Supercritical fluid extraction and fractionation of natural matter. J. Supercrit. Fluids 38, 146–166.

Sartori, J.A.S., Scalise, C., Baptista, A.S., Lima, R.B., Aguiar, C.L., 2013. Parameters of influence on extraction of phenolic compounds from sugarcane tops with total antioxidante activity. Biosci. J. 29, 297–307.

Shaidi, F., Naczk, M., 1995. Food Phenolics: Sources, Chemistry, Effects and Applications. Technomic Publishing, Lancaster, pp. 281–319.

Singleton, V.L., Orthofer, R., LAMUELA-Raventós, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Souza, A.D., Maia, A.I.V., Rodrigues, T.H.S., Canuto, K.M., Ribeiro, P.R.V., Pereira, R.C.A., Vieira, R.F., Brito, E.S., 2016. Ultrasound-assisted and pressurized liquid extraction of phenolic compounds from *Phyllanthus amarus* and its composition evaluation by UPLC-QTOF. Ind. Crops Prod. 79, 91–103 2016.

Storck, C.R., Nunes, G.L., Oliveira, B.B., Basso, C., 2013. Leaves, stems, bark and seeds of vegetables: nutritional composition, use in feeding and sensory analysis of preparations. Ciência Rural. 43, 537–543.

Toma, M., Vinatoru, M., Paniwnyk, L., Mason, T.J., 2001. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. Ultrason. Sonochem. 8, 137–142.

US-FDA, 2016. United States – Food and Drug Administration. Available at: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?Fr=184.1240, Accessed date: 13 April 2017.

Vaccari, G., Tamburini, E., Sgualdino, G., Urbaniec, K., Klemeš, J., 2005. Overview of the environmental problems in beet sugar processing: possible solutions. J. Clean. Prod. 13, 499–507.

Yilmaz, Y., Toledo, R.T., 2006. Oxygen radical absorbance capacities of grape/wine industry byproducts and effect of solvent type on extraction of grape seed polyphenols. J. Food Compos. Anal. 19, 41–44.

Zetzl, C., Brunner, G., Meireles, M.A.A., 2003. Standardized low-cost batch SFE Units for University education and comparative research. In: Proceedings of the 6th International Symposium on Supercritical Fluids, vol. 1. pp. 577–581.