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Antioxidant Activity of Extracts of Soursop (*Annona muricata* L.) Leaves, Fruit Pulps, Peels, and Seeds

Hakime Hülya Orak[1]*, Ilayda Sevik Bahrisefit[2], Temine Sabudak[2]

[1]Department of Food Technology, Vocational School of Technical Sciences, Namik Kemal University, 59030 Tekirdağ, Turkey
[2]Department of Chemistry, Faculty of Science and Arts, Namik Kemal University, 59030 Tekirdağ, Turkey

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The total phenolic content (TPC), total flavonoid content (TFC), and the antioxidant activity of soursop (*Annona muricata* L.) leaf, fruit pulp, seed, and peel extracts obtained using successive extraction with hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) were determined. The Hxn soursop seed extract was analysed by GC-MS. The highest TPC was determined in MeOH extracts. MeOH and EtOAc extracts were rich sources of flavonoids. Generally, soursop leaf and fruit pulp extracts had the highest and the lowest both TPC and TFC, respectively. Fatty acids were dominant in the Hxn seed extract. Among antioxidants, terpenoids ((*E*)-nerolidolas as dominant) and phytosterols ((3-β)-stigmast-5--en-3-ol with high content) were identified. The soursop seed, followed by leaf and peel extracts (MeOH and EtOAc) had the highest DPPH$^{\bullet}$ scavenging activity, TEAC, FRAP, and CUPRAC. Antioxidant activity of peel extracts (MeOH and EtOAc) was particularly high in β-carotene-linoleic acid emulsion system. Strong correlations were found between TPC, TFC, TEAC, FRAP, and results of DPPH assay. In conclusion, soursop leaves and fruit seeds and peels, which are cheap, waste plant material, could be considered as a source of phenolic antioxidants with a high antioxidant activity.

INTRODUCTION

In recent years, tropical and exotic fruits have been in the focus of researchers interest. Consumer interest in them increases as well. This is due to the potential health benefits of many tropical and exotic plants. One of them is soursop (*Annona muricata* L.), commonly called graviola, belonging to the *Annonacea* family. Soursop is native to the warmest areas of South and North America and is now widely distributed throughout tropical and subtropical regions of Central and South America, Western Africa, and Southeast Asia [Moghadamtousi *et al.*, 2015; Coria-Tellez *et al.,* 2018]. The soursop fruits are quite large (15–20 cm). The pulp contains 55–170 black seeds covered with green peel. Peels and seeds are inedible parts of soursop fruit, there is a high amount of by-products from this fruit that have not been studied as a source of bioactive compounds [Aguilar Hernandez *et al.,* 2019]. However, in recent years, interest in the utilization of fruit and vegetable by-products has increased due to the potential high content of nutrients and bioactive compounds, such as phenolics, dietary fiber, and vitamins, among others [Kosińska *et al*., 2012; Sagar *et al*., 2018; Kuchtová *et al.*, 2018]. The exotic fruit by-products have previously been considered as a source of valuable food additives of natural origin [Ayala-Zavala *et al.*, 2011].

Aromatic soursop fruits are readily used culinary. Pulp is consumed raw and is used to prepare juice, ice-cream or jelly [Benites *et al.*, 2015]. Moreover, different parts of soursop (leaf, bark, root, fruit, and seed) are used in traditional medicine against several ailments including hypertension, inflammation, diabetes, gastrointestinal disorders, respiratory diseases, and cancers [Coria-Tellez *et al.,* 2018; Chamcheu *et al.,* 2018]. The medicinal activities and the health benefits of *A. muricata* L. have been attributed to their phytochemicals including acetogenins, alkaloids, megastigmanes, phenolics, cyclopeptides, and essential oils [Moghadamtousi *et al.,* 2015].

The phenolic compounds are the major phytochemicals responsible for the antioxidant potential of soursop leaves and fruits [Coria-Tellez *et al*., 2018]. Among them, the phenolic acids (mainly hydroxycinnamic acids), flavonoids, and tannins (including procyanidin dimers) were determined in *A. muricata* L. leaves, pulp, and seeds [Marques & Farah, 2009; Huang *et al.*, 2010; Nawwar *et al.*, 2012; Jiménez *et al.*, 2014; Nam *et al.*, 2017]. Solvent extractions are commonly used to obtain plant extracts with phenolic compounds. These conventional techniques were also applied to soursop materials [da Silva *et al.*, 2014; Nam *et al.*, 2017]. The polarity of the solvent is one of the important parameters of the extraction process. There are some reports indicating that the type of solvent affected the bioactivity of *A. muricata* extracts [George *et al.*, 2015; Chamcheu *et al.*, 2018]. Generally, hexane and petroleum ether are suitable for the extraction

* Corresponding Author: E-mail: horak@nku.edu.tr (H.H. Orak)

of phenolic terpenes. Ethyl acetate is used for the extraction of low-molecular-weight phenolics (phenolic acids and flavonoid aglycons). Methanol, ethanol and their mixtures with water allowed extracting high-molecular-weight phenolics and flavonoid glycosides [Oreopoulou & Tzia, 2007].

The aim of our study was to compare extracts obtained by solvents with increasing polarity from soursop fruit pulps, fruit by-products (seeds and peels), and leaves in terms of their total phenolic and flavonoid contents and their antioxidant activity in polar and lipid emulsion systems. Additionally, the antioxidants of hexane seed extract were looked for using GC-MS analysis.

MATERIAL AND METHODS

Plant material

Leaves and mature fruits of soursop (*A. muricata* L.) were obtained from the Dominica Island in December 2017. Nine fruits were sampled at about 0.6–1.4 kg weight. The soursop fruits were harvested from natural grown trees in the Dominica Island and transferred by plane. The fruits were processed for analysis four days after harvest. Peels (Pl), seeds (S), and pulp (P) were manually separated from fruits. All parts of the fruits as well as leaves (L) were frozen at -40°C and dried using a vacuum freeze dryer (FT 33; Armfield, Ringwood, UK).

Extracts preparation

Dried plant materials were grounded and subjected to successive extraction with solvents of increasing polarity. Hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) were used one after the other. Extraction was carried out for twelve hours at room temperature with pure solvent by using Soxhlet extraction method. The ratio of weights of plant material to the solvent was 1:3. Solvents were evaporated under vacuum (R-210 Rotavapor, B-491 heating bath, V-710 vacuum pump; Büchi Labortechnik, Flawil, Switzerland). Samples were stored at -22°C until analysed.

Total phenolics content (TPC)

The content of total phenolics of soursop extracts was evaluated using Folin-Ciocalteu's reagent. The absorbance of reaction mixtures was read at 725 nm (Hitachi U-2000 spectrophotometer 1210002, Tokyo, Japan) [Amarowicz *et al.,* 2004]. The TPC was expressed as mg (+)-catechin equivalents (CE) per g of extract.

Total flavonoids content (TFC)

The content of total flavonoids of soursop extracts was determined according to the procedure described by Zhishen *et al.* [1999]. The extract (250 μL, concentration of 1–10 mg/mL depending on solvent used) was mixed with distilled water (1.25 mL) and sodium nitrite solution (5%, 75 μL). After 6 min of incubation, aluminium chloride (10%, 150 μL) was added to the mixture followed by sodium hydroxide (1 M, 500 μL). Samples were immediately diluted with distilled water (2.5 mL). The absorbance was measured at 510 nm. The TFC was expressed as mg (+)-catechin equivalents (CE) per g of extract.

Antioxidant activities of soursop extracts

DPPH$^{\bullet}$ scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) scavenging activity of soursop extracts was determined by the method of Brand-Williams *et al.* [1995]. Firstly, the methanol (2 mL) and methanolic solution of 1 mM DPPH radicals (0.25 mL) were mixed. Then, extracts (0.1 mL) in different concentrations (0.4–2.0 mg/assay) were added. After the reaction in dark (20 min), the absorbance was measured at 517 nm. The EC_{50} value (the half-maximal effective concentration) was determined on the basis of the plot of absorbance *vs.* extract concentrations.

Trolox equivalent antioxidant capacity (TEAC)

Re *et al.* [1999] method was used to determine TEAC. The portions of 2 mL of [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation (ABTS$^{\bullet+}$) reagent and 20 μL of soursop extracts (from 1–2 mg/mL extract concentrations) were mixed and incubated at 30°C for 6 min. The absorbance of samples was determined at 734 nm and the results were expressed as mmol Trolox equivalents per g of extract.

Ferric-reducing antioxidant power (FRAP)

The FRAP assay was carried out according to Benzie & Strain [1996] procedure. The reaction was performed by mixing the extract solution (75 μL), distilled water (225 μL), and FRAP solution (2.25 mL). The FRAP solution was prepared by mixing 2,4,6-Tri(2-pyridyl)-*s*-triazine (10 mM in 40 mM HCl; 6 mL), acetate buffer (300 mM; pH 3.6; 60 mL), and ferric chloride (20 mM; 6 mL). The mixture was incubated at 37°C (for 30 min) and the absorbance was measured at 593 nm. Ferrous sulfate was used to prepare calibration curve and the results were evaluated as μmol Fe^{2+}equivalents per g of extract.

Cupric ion-reducing antioxidant capacity (CUPRAC)

CUPRAC assay was performed according to Apak *et al.* [2004] method. For determination of the antioxidant activity of soursop extracts, 0.5 mL of $CuCl_2$ solution (10 mM), 0.5 mL of neocuproine ethanolic solution (7.5 mM), 0.5 mL of ammonium acetate buffer (1 M; pH7.0), and 0.25 mL of extract solutions (1–2 mg/mL extract concentrations) were added to the test tubes. The volume of the reaction mixtures was adjusted to 2.05 mL with water. Well-mixed tubes were closed and incubated (30 min at ambient temperature). Absorbance readings were done at 450 nm. The results were calculated based on the calibration curve obtained for Trolox and expressed as mmol Trolox equivalents per g of extract.

β-Carotene-linoleic acid bleaching

The β-carotene-linoleic acid emulsion oxidation was carried out according to Miller [1971] procedure with modifications [Orak *et al.*, 2019]. Firstly, the β-carotene (1.0 mg) was dissolved in chloroform (5 mL). Then, Tween40 (400 mg) and linoleic acid (40 μL) were added. The chloroform was evaporated and water (25 mL) was added to the residue with vigorous stirring. For antioxidant activity measurement,

the emulsion (250 μL) was vortexed with extract solution or standard antioxidant (butylated hydroxyanisole, BHA) solution (100 μL collected from 1 mg/mL concentration). The oxidation reaction temperature was 42°C, the absorbance of samples was monitored in 30 min intervals throughout 180 min at 470 nm. The percentage of non-oxidized β-carotene after 180 min of emulsion oxidation was calculated.

GC-MS analysis

GC-MS analysis was done using the HP 6890 instrument (Hewlett-Packard, Palo Alto, CA, USA) combined with a mass selective detector (GCMS-QP2010 Ultra Shimadzu, Kioto, Japan). The HP-5MS capillary column (5% phenyl methyl siloxane, 30 m × 250 μm, film thickness 0.25 μm, Agilent, Palo Alto, CA, USA) was used. Helium was used as a carrier gas. Its flow rate was 1.0 mL/min. The column initial temperature was 180°C (1 min after injection). The temperature increased to 250°C with an 8°C/min heating ramp in a 1 min holding time, and increased to 300°C with 2°C/min heating ramp in 10 min. The injections (5 μL) were done in the split mode with a split ratio of 10:1. For the analysis, the 250°C was interface temperature, the 280°C was injector temperature and running time was 49 min. MS scan range was m/z 20–440 using electron impact (EI) ionization (70 eV) and an ion source temperature of 250°C. Components were identified according to the comparison of their mass spectra with those of Wiley 9 and NIST library. The relative percentage of separated compounds was determined from Total Ion Chromatogram by the computerized integrator.

Statistical analysis

The MSTAT-C software package was used for statistical analyses. The results were subjected to ANOVA with a Fisher's Least Significant Difference (LSD) post hoc test ($p<0.05$). Moreover, the correlations between variables were determined and Pearson correlation coefficients (r) were calculated.

RESULTS AND DISCUSSION

Extraction yield, total phenolic and total flavonoid contents

The yields of hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH) extracts of leaves (L), fruit pulp (P), seeds (S), and peels (Pl) of soursop were between 0.23% and 64.14% (Table 1). The largest yield was obtained for P-MeOH extract (64.14%). MeOH was also the most effective solvent for peels (16.50%) and leaves (12.07%). Hxn was able to extract the largest amount of matter from seeds (24.26%). Yields of MeOH extracts determined in our study were in line with those reported for methanol-water extracts of seeds and pulps of some *Annona* species fruits; *e.g.*, *A. coriacea* L. (14.5% and 20.5%, respectively) and *A. sylvatica* L. (8.7% and 5.2%, respectively) [Benites *et al.,* 2015] as well as for methanolic extract of *A. muricata* L. leaves (10.30%) [Nam *et al.,* 2017].

The TPC varied in the range of 10.92–244.61 mg CE/g in leaf extracts; 20.75–187.48 mg CE/g in peel extracts; 19.84–50.15 mg CE/g in pulp extracts; and 5.06–202.17 mg CE/g in seed extracts (Table 1). MeOH extracts had a much higher TPC than the extracts obtained using other solvents (except fruit pulp extracts). In the case of fruit pulp, EtOAc was a more effective ($p<0.05$) phenolic compound extractant. Hxn extracts had the lowest TPC ($p<0.05$). Converting TPC of extracts by extraction yields, it can be noted that peels and leaves were the richest sources of phenolic compounds, followed by pulp and seeds. Higher TPC of soursop fruit pulp compared to that of seeds was in line with literature data [da Silva *et al.*, 2014]. Moreover, higher TPC in the peels than in the pulp of fruits of different *Annona* species (*A. cherimola* L. and *A. squamosal* L.) was previously reported [Loizzo *et al.*, 2012; Huang *et al.*, 2010].

The TFC of extracts is shown in Table 1. The highest TFC was determined in L-MeOH extract (81.32 mg/CEg) and the lowest one in S-Hxn extract (1.54 mg CE/g). Generally, TFC of fruit pulp and by-products decreased in the following order L>S≥Pl>P. When the results were compared based on the extraction solvent used, MeOH and EtOAc extracts had the highest TFC. On the other hand, as could be expected, Hxn was the least effective solvent for flavonoid extraction. Loizzo *et al.* [2012] reported that TFC/TPC ratios of *Annona* fruit peel and pulp ranged from 0.3 to 0.6. In our study, similar values were obtained for MeOH extracts, but TFC/TPC ratios of EtOAc extracts were significantly higher, *i.e.* at about 0.9. This indicates good selectivity of EtOAc for flavonoid extraction from soursop fruits

TABLE 1. The extract yield, total phenolic content (TPC) and total flavonoid content (TFC) of soursop (*A. muricata* L.) leaves (L), fruit pulp (P), peels (Pl) and seeds (S) extracts obtained using hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH).

Extract	Extract yield (%)	TPC (mg CE/g)	TFC (mg CE/g)
L-Hxn	3.66	10.92 ± 1.28^{i}	2.62 ± 0.19^{i}
L-DCM	1.10	30.60 ± 2.71^{f}	26.46 ± 1.57^{e}
L-EtOAc	0.83	73.42 ± 3.48^{d}	65.98 ± 4.79^{b}
L-MeOH	12.07	244.61 ± 7.00^{a}	81.32 ± 3.45^{a}
Pl-Hxn	0.59	20.75 ± 0.20^{h}	1.68 ± 0.09^{j}
Pl-DCM	0.23	27.35 ± 0.50^{g}	15.77 ± 0.22^{f}
Pl-EtOAc	0.25	56.33 ± 4.97^{e}	50.22 ± 2.90^{c}
Pl-MeOH	16.50	187.48 ± 6.78^{c}	36.10 ± 1.04^{cd}
P-Hxn	0.71	19.84 ± 0.90^{h}	2.16 ± 0.12^{i}
P-DCM	0.25	26.23 ± 0.96^{g}	13.34 ± 0.28^{g}
P-EtOAc	0.26	50.15 ± 4.57^{e}	34.41 ± 2.20^{d}
P-MeOH	64.14	38.36 ± 2.12^{f}	13.95 ± 0.19^{g}
S-Hxn	24.26	5.06 ± 1.37^{j}	1.54 ± 0.08^{i}
S-DCM	3.01	20.70 ± 5.00^{h}	11.45 ± 0.59^{h}
S-EtOAc	0.58	53.73 ± 2.81^{e}	48.04 ± 2.11^{c}
S-MeOH	3.66	202.17 ± 12.99^{b}	56.59 ± 5.29^{c}

Data are expressed as the mean ± standard deviation for each extract (n=3). Values in the same column having different superscript letters differ significantly ($p< 0.05$). CE: catechin equivalents.

and leaves. In previous studies, the presence of flavonoids belonging to subclasses of flavan-3-ols and flavonols was determined in soursop leaves, fruit pulp, and peels [Huang *et al.*, 2010; Nawwar *et al.*, 2012; Jiménez *et al.*, 2014; Nam *et al.*, 2017]. Besides flavonoids, hydroxycinnamic acid derivatives were identified in leaves and pulp [Marques & Farah, 2009; Jiménez *et al.*, 2014; Nam *et al.*, 2017]. In turn, phenolic terpenoids were found in soursop seeds [Huang *et al.*, 2010].

Antioxidant activity of soursop leaf and fruit part extracts

Five assays in which antioxidants act as free radical scavengers (TEAC and DPPH assay), as reducing agents (FRAP and CUPRAC) or as inhibitors of the lipid substrate oxidation (β-carotene-linoleic acid bleaching assay) were used to determine the antioxidant activities of extracts of soursop leaves and fruit pulps, peels and seeds.

The DPPH$^{\bullet}$ scavenging activity of the soursop extracts was expressed as EC_{50} values. The results are presented in Table 2. The highest antiradical activity against DPPH$^{\bullet}$ with the lowest EC_{50} value had the S-MeOH extract (0.044 mg/mL). The lowest antiradical activity was determined for the P-Hxn extract (EC_{50} 0.411 mg/mL). In addition to S-MeOH, other methanolic extracts were also characterized by low EC_{50} values, especially in the case of leaf (0.063 mg/mL) and peel (0.090 mg/mL). The extract obtained with use of ethyl acetate and dichloromethane had intermediate EC_{50} values for each of the plant materials except peels where antiradical activity of Pl-Hxn and Pl-DCM extracts as well as Pl-DCM and Pl-EtOAc extracts did not differ significantly ($p>0.05$). Given the type of extracted material, the DPPH$^{\bullet}$ scavenging activity decreased generally in the following order: S>L>Pl>P. The TEAC values shown in Table 2 indicate the ability of soursop extract to inactivate ABTS$^{\bullet+}$. Compared to DPPH$^{\bullet}$ scavenging activity, the highest TEAC was determined for S-MeOH (0.905 mmol Trolox/g) and L-MeOH (0.848 mmol Trolox/g) extracts. Moreover S-EtOAc (0.572 mmol Trolox/g), L-EtOAc (0.474 mmol Trolox/mg), and Pl-MeOH (0.438 mmol Trolox/g) extracts had high TEAC. Plant materials could be ordered as follows: S ≥ L > Pl >P, if decreasing TEAC values of MeOH and EtOAc extracts were considered.

TABLE 2. Ferric-reducing antioxidant power (FRAP), Trolox equivalent antioxidant capacity (TEAC) and DPPH$^{\bullet}$ scavenging activity of soursop (*A. muricata* L.) leaves (L), peels (Pl), fruit pulp (P), and seeds (S) extracts obtained using hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH).

Extract	TEAC (mmolTrolox/g)	FRAP (μmol Fe^{2+}/g)	EC_{50} DPPH (mg/mL)
L-Hxn	0.222±0.029^{i}	66.5±0.48^{l}	0.312±0.04^{c}
L-DCM	0.242±0.008^{hi}	104.0±1.7^{ij}	0.143±0.02^{h}
L-EtOAc	0.474±0.009^{d}	339.7±5.9^{e}	0.136±0.06^{h}
L-MeOH	0.848±0.011^{b}	798.9±2.4^{b}	0.063±0.04^{k}
Pl-Hxn	0.251±0.018^{h}	102.8±4.3^{j}	0.264±0.02^{e}
Pl-DCM	0.253±0.014^{h}	180.7±4.2^{h}	0.286 ±0.02^{d}
Pl-EtOAc	0.300±0.005^{f}	284.1±6.8^{f}	0.277±0.10^{de}
Pl-MeOH	0.438±0.005^{e}	465.2±8.0^{c}	0.090±0.05^{j}
P-Hxn	0.180±0.012^{j}	75.9±3.0^{k}	0.411±0.03^{a}
P-DCM	0.225±0.029^{i}	117.9±1.8^{i}	0.328±0.05^{b}
P-EtOAc	0.280±0.002^{g}	210.4±9.6^{g}	0.307±0.02^{c}
P-MeOH	0.104±0.002^{k}	97.0±2.1^{j}	0.281±0.04^{d}
S-Hxn	0.110±0.009^{kj}	33.2±4.2^{m}	0.231±0.02^{f}
S-DCM	0.201±0.006^{ij}	77.5±3.2^{k}	0.191±0.04^{g}
S-EtOAc	0.572±0.025^{c}	447.4±3.7^{d}	0.115±0.02^{i}
S-MeOH	0.905±0.029^{a}	1100.6± 9.3^{a}	0.044±0.02^{l}

Data are expressed as the mean ± standard deviation for each extract (n=3). Values in the same column having different letters differ significantly ($p < 0.05$).

The ability of extracts to reduce Fe^{3+} (FRAP) and Cu^{2+} (CUPRAC) is shown in Table 2 and Figure 1, respectively. The FRAP ranged from 33.2 to 1100.6 mmol Fe^{2+}/g in seed

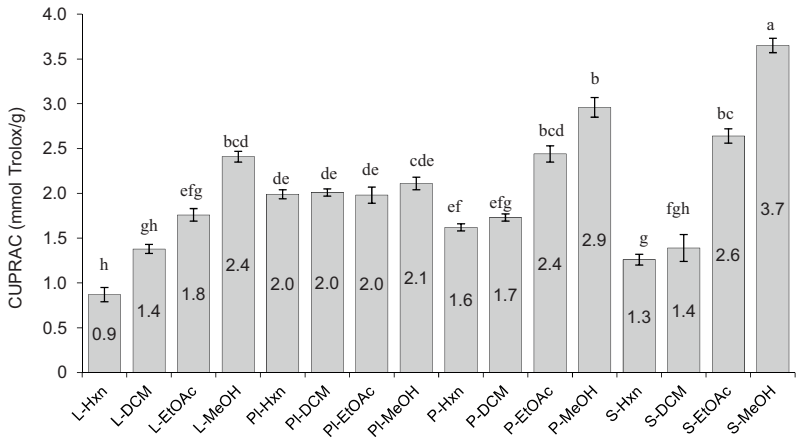

FIGURE 1. Cupric ion reducing antioxidant capacity (CUPRAC) of soursop (*A. muricata* L.) leaves (L), fruit pulp (P), peels (Pl), and seeds (S) extracts obtained using hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH). Data are expressed as mean ± standard deviation (n=3) for each extract. Bars having different letters differ significantly (p<0.05).

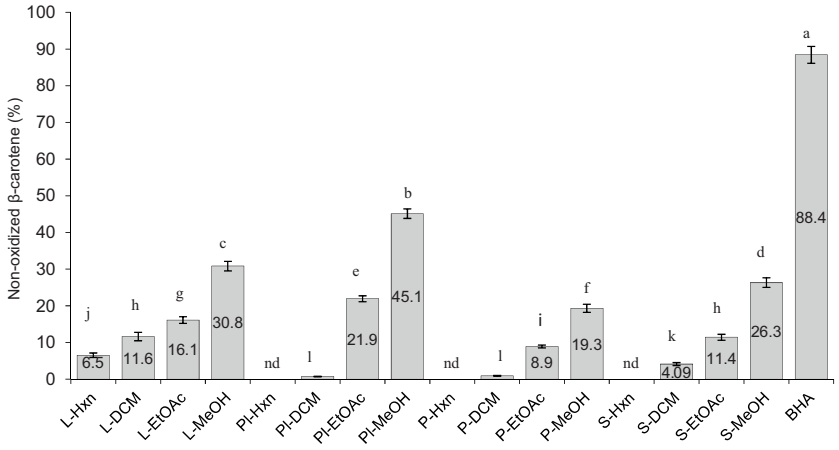

FIGURE 2. Inhibition of β-carotene-linoleic acid emulsion oxidation by soursop (*A. muricata* L.) leaves (L), fruit pulp (P), peels (Pl), and seeds (S) extracts obtained using hexane (Hxn), dichloromethane (DCM), ethyl acetate (EtOAc), and methanol (MeOH). Data are expressed as mean ± standard deviation (n = 3) for each extract. Bars having different letters differ significantly ($p<0.05$); nd – not detected.

extracts, from 75.9 to 210.4 μmol Fe^{2+}/g in pulp extracts, from 102.8 to 465.2 mmol Fe^{2+}/g in peel extracts, and from 66.4 to 798.9 mmol Fe^{2+}/g in leaf extracts. The differences between CUPRAC of soursop extracts were significant ($p<0.05$). The values ranged from 0.87 mmol Trolox/g to 3.65 mmol Trolox/g. In both assays, again, the S-MeOH and L-MeOH extracts exhibited the highest activity and hexane was the least effective in the extraction of compounds with the ability to reduce metal ions.

Antioxidant activity of soursop extracts determined in the β-carotene-linoleic acid emulsion system is shown in Figure 2. The results are slightly different from those obtained in the previously discussed assays, because after 180 min of oxidation, the most of non-oxidised β-carotene (45.1%) remained in Pl-MeOH extract. Among the EtOAc extracts, the peel extract also had the highest ability to inhibit emulsion oxidation. However, the antioxidant activity of L-MeOH and S-MeOH extracts was also high; the extracts inhibited β-carotene oxidation at 30.8% and 26.3%, respectively. In turn, all Hxn and some of DCM extracts were not able to inhibit the oxidation of the emulsion. All extracts showed a lower antioxidant activity than BHA.

The higher antioxidant activity of soursop seed extracts (MeOH and EtOAc) compared to pulp extracts determined in our studies in all used assays was in line with Benite *et al*. [2015] report in which ABTS, DPPH and β-carotene--linoleic acid bleaching assays of soursop seed and pulp methanol-water extracts were carried out. In turn, Loizzo *et al.* [2012] found that ethanolic extract from *A. cherimola* L. peel had higher FRAP, DPPH$^{\bullet}$ scavenging activity and ability to inhibit oxidation of β-carotene-linoleic acid emulsion than extract from pulp which is also accordance with our finding. However, in mentioned study the significant difference between ABTS results for peel and pulp extracts was not noted.

The results of correlation analysis are shown in Table 3. TPC of extracts of soursop leaves and fruit parts was significantly correlated ($p<0.05$) with TFC ($r=0.761$) as well as with results of antioxidant assays, especially with FRAP ($r=0.899$), TEAC ($r=0.872$), and emulsion oxidation ($r=0.865$). Weaker correlation was noted only between TPC and CUPRAC ($r=0.589$). The correlations of TFC with FRAP, TEAC, and results of emulsion oxidation were also significant ($p<0.05$), and confirmed by high correlation coefficients – 0.900, 0.887 and 0.713, respectively. In a previous study, strong correlations between TPC and antioxidant activities determined by FRAP and DPPH assays were reported for soursop leaf extracts obtained with using different solvents [George *et al.*, 2015]. In turn, Nam *et al.* [2017] found that r values of correlations between TPC and antiox-

TABLE 3. Pearson's correlation coefficients (r) between total phenolic content (TPC), total flavonoid content (TFC), and results of antioxidant assays of extracts of soursop (*A. muricata* L.) leaves and fruit pulp, peel and seed.

	TPC	FRAP	TEAC	CUPRAC	DPPH$^{\bullet}$ (EC_{50})	Emulsion oxidation
TFC	0.761	0.900	0.887	0.646	-0.680	0.713
TPC	1	0.899	0.872	0.589	-0.719	0.865
FRAP		1	0.968	0.724	-0.753	0.739
TEAC			1	0.731	-0.807	0.640
CUPRAC				1	-0.655	0.388
DPPH$^{\bullet}$ (EC_{50})					1	-0.477

FRAP: ferric-reducing antioxidant power; TEAC:Trolox equivalent antioxidant capacity; CUPRAC: cupric ion reducing antioxidant capacity.

TABLE 4. Chemical compounds of soursop (*A. muricata* L.) seed hexane extract identified by GC-MS.

Peak	RT	Compound	%
1	8.83	Decane	0.57
2	12.41	Undecane	0.34
3	16.12	Tridecane	0.45
4	18.29	(*E*)-2-Decenal	1.28
5	19.43	(*E*,*E*)-2,4-Decadienal	3.23
6	23.18	Tetradecane	0.55
7	26.46	Pentadecane	0.14
8	26.78	β-Bisabolene	0.83
9	27.26	β-Sesquiphellandrene	0.53
10	28.46	(*E*)-Nerolidol	3.62
11	29.33	1-Heptadecene	0.24
12	29.57	*n*-Octadecane	0.34
13	32.52	Heptadecane	0.15
14	34.16	Tetradecanoic acid	0.29
15	35.33	Nonadecane	0.13
16	38.01	2-Nonadecanon	0.15
17	38.65	Hexadecenoic acid, methyl ester	0.21
18	39.69	Pentadecanoic acid	19.92
19	40.39	1-Nonadecene	1.17
20	41.04	Hexadecanal	0.26
21	41.99	Heptadecanoic acid	0.16
22	42.83	9,12-Octadecadienoic acid(*Z*,*Z*), methyl ester	0.17
23	42.99	6-Octadecenoic acid, methyl ester	0.31
24	43.89	9,12-Octadecadienoic acid (*Z*,*Z*)	15.58
25	44.06	9-Octadecenoic acid	27.82
26	44.47	Octadecanoic acid	3.40
27	44.59	(*Z*)-9-Octadecenoic acid, ethyl ester	1.70
28	44.84	Hexadecanamide	0.64
29	45.01	Hexadecanoic acid, butyl ester	0.20
30	45.20	1-Nonadecene	0.94
31	45.88	Octadecanal	0.23
32	47.57	Heneicosane	0.20
33	48.72	6,9-Octadecadienoic acid, methyl ester	0.39
34	48.84	(*Z*)-9-Octadecenamide	3.04
35	49.38	Octadecanamide	0.20
36	49.61	1-Eicosanol	0.61
37	51.31	Di-(9-octadecenoyl)-glycerol	0.13
38	53.04	Phthalic acid mono-2-ethylhexyl ester	0.27
39	53.40	Oxirane, hexadecyl	0.27
40	56.12	Humulane-1,6-dien-3-ol	0.52
41	57.18	Glyceryl trioleate	0.67
42	58.39	(3-β)-Stigmast-5-en-3-ol	7.45
43	59.29	Bis (2-ethylhexyl) phthalate	0.34
44	59.90	Urs-12-ene	0.28

RT: Retention times

TABLE 5. The chemical class distribution of the compounds of soursop (*A. muricata* L.) seed hexane extract.

Chemical class of compounds	Distribution (%)
Fatty acids	67.17
Unsaturated fatty acids	43.40
Saturated fatty acids	23.77
Terpenoids	13.23
Alkanes	2.87
Alkenes	2.35
Aldehydes and ketones	5.15
Alcohols	0.74
Esters	4.26
Amides	3.88
Epoxides	0.27
Total	99.92

idant activity (FRAP, ABTS, and DPPH assays) of extracts of different parts of *A. muricata* L. were higher compared to those determined for the TFC – antioxidant activity correlation. In the present study, the FRAP, TEAC, and CUPRAC values were significantly ($p<0.05$) correlated with each other, wherein the highest r value (0.968) was noted for TEAC and FRAP correlation (Table 3). Strong, negative correlations were found between EC_{50} values of DPPH assay and FRAP and TEAC. This finding was in line with literature data [Nam *et al.*, 2017]. Additionally, the lower r value was determined for correlations between emulsion oxidation results and results of CUPRAC ($r=0.388$) and DPPH assay ($r=-0.477$) (Table 3).

GC-MS analysis of hexane extract of soursop seeds

The hexane extract of soursop seeds was obtained with a high yield (Table 1). The TPC and TFC of this extract were low. Despite this, it showed some antioxidant activity. Therefore, GC-MS analysis of hexane seed extract was carried out in search of potential antioxidants.

The GC-MS analysis allowed identifying 44 compounds in the hexane extract. These compounds were characterized by their retention time (RT), their molecular formula, and contents which were calculated based on peak area (%) (Table 4). According to chemical class distribution, fatty acids were most abundant (67.17%), followed by terpenoids (13.23%), aliphatic hydrocarbons (alkanes/alkenes) (5.22%), aldehydes and ketones (5.15%), esters (4.26%), alcohols (0.74%), and amides (3.88%) (Table 5). Unsaturated fatty acids constituted 43.40% of all determined compounds. The content of saturated fatty acids was 23.77%. Oleic acid (9-octadecenoic acid; 27.82 %) and linoleic acid (9,12-octadecadienoic acid; 15.58%) were the major unsaturated fatty acids in the soursop seed hexane extract. Especially, linoleic acid is known as an essential fatty acid with an important metabolic role [Eromosele & Eromosele, 2002]. The high

content of oleic and linoleic acids in the hexane seed extract confirmed previous findings. Both acids were found as dominant in *A. muricata* L. seed oil [da Silva & Jorge, 2017; Pinto *et al.*, 2018]. In turn, the percentage of stearic acid (pentadecanoic acid) in total fatty acids was low compared to the result presented in our study (19.92%). Among phytosterols, the content of 3-β-stigmast-5-en-3-ol (7.45%) was the highest in the hexane extract (Table 4). da Silva & Jorge [2017] noted that this compound was the major phytosterol of soursop seed oil. The antioxidant activity of 3-β-stigmast-5-en-3-ol examined both *in vitro* (DPPH and ABTS assays) and *in vivo* had already been reported [Ayaz *et al*., 2017]. Its anti-proliferative properties were noted as well [Moon *et al.,* 2008]. Terpenoids are another class of compounds with recognized antioxidant activity; they were detected in the analysed soursop hexane seed extract. The main terpenoid in the extract was (*E*)-nerolidol (Table 4). Chan *et al.* [2016] reviewed various biological activities of this sesquiterpene alcohol, including its antioxidant activity. The major aldehydes in the extract were identified as (*E*)-2-decenal (1.28%) and (*E*,*E*)-2,4-decadienal (3.23%). Caboni *et al.* [2012] reported a high nematicidal activity of both compounds. In turn, Cheng *et al.* [2008] suggested antioxidant activity of (*Z*)-9-octadecenamide, which was also present in soursop seed hexane extract (Table 4).

CONCLUSIONS

The successive extraction of soursop (*Annona muricata* L.) leaves, fruit pulps, seeds, and peels with hexane, dichloromethane, ethyl acetate, and methanol allowed obtaining extracts with different antioxidant activity. More polar solvents were better extractants of antioxidants; methanol extracts were characterised by the highest total phenolics content while both methanol and ethyl acetate extracts were rich sources of flavonoids. Considering each plant material individually (leaves, seeds, and peels), the methanol and ethyl acetate extracts had the highest antioxidant activity examined as antiradical activity (TEAC and DPPH assay), as ability to reduce metal ions (FRAP and CUPRAC), and as ability to inhibit β-carotene-linoleic acid emulsion oxidation. Besides, while ethyl acetate was a good solvent for the extraction of antioxidants from pulp and peel, methanol was better for leaves and seeds. Low total phenolics and especially total flavonoids contents of the hexane extract was correlated with their low antioxidant activity. However, bioactive constituents of the hexane seed extract, such as terpenoids and phytosterols, could positively influence its antioxidant activity. (*E*)-Nerolidol and (3-β)-stigmast-5-en-3-ol, both with previously recognised antioxidant activity, were determined in the hexane seed extract as major terpenoid and phytosterol, respectively.

When considering the total phenolic and flavonoid contents of extracts, the best material turned out to be leaves and seeds. Methanol and ethyl acetate extracts of these materials had a high antioxidant activity in non-lipid assays (DPPH$^{\bullet}$ scavenging activity, TEAC, FRAP, and CUPRAC). Antioxidant activity of peel extracts was particularly high in the β-carotene-linoleic acid emulsion system. Pulp extracts showed the lowest antioxidant activity with lower total phenolic and flavonoid contents. Strong correlations were found between total phenolic and flavonoid contents and antioxidant activity determined as TEAC, FRAP, and DPPH$^{\bullet}$ scavenging activity.

Our study showed that soursop leaves and soursop by-products from fruit processing (seeds and peels) have the potential to be used to obtain extracts with a high antioxidant activity.

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CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

REFERENCES

1. Aguilar-Hernandez, G., Garcia-Magana, M.L., Vivar-Vera, M.A., Sayago-Ayerdi, S.G., Sanchez-Burgos, J.A., Morales-Castro, J., Anaya-Esparza, L.M., Gonzalez, E.M. (2019). Optimisation of ultrasound-assisted extraction of phenolic compounds from *Annona muricata* by-products and pulp. *Molecules, 24*(5), art. no. 905.
2. Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamin C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, *52*(26), 7970–7981.
3. Amarowicz, R., Pegg, R.B., Rahimi-Moghaddam, P., Barl, B., Weil, J.L. (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*, *84*(4), 551–562.
4. Ayala-Zavala, J.F., Vega-Vega, V., Rosas-Domínguez, C., Palafox-Carlos, H., Villa-Rodriguez, J.A., Wasim Siddiqui, Md., Dávila-Aviña, J.E., González-Aguilar, G.A. (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Research International*, *44*(7), 1866–1874.
5. Ayaz, M., Junaid, M., Ullah, F., Subhan, F., Sadiq, A., Ali, G., Ovais, M., Shahid, M., Ahmad, A., Wadood, A., El-Shazly, M., Ahmad, N., Ahmad, S. (2017). Anti-Alzheimer's studies on beta-sitosterol isolated from *Polygonum hydropiper* L. *Frontiers in Pharmacology*, *8*, art. no. 697.
6. Benites, R.S.R., Formagio, A.S.N., Argandoña, E.J.S., Volobuff, C.R.F., Trevizan, N.F., Vieira, M.C., Silva, M.S. (2015). Contents of constituents and antioxidant activity of seed and pulp extracts of *Annona coriacea* and *Annona sylvatica*. *Brazilian Journal of Biology, 75*(3), 685–691.
7. Benzie, I.F.F., Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry, 239*(1), 70–76.
8. Brand-Williams, W., Cavalier, M.E., Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, *28*(1), 25–30.

9. Caboni, P., Ntalli, N.G., Aissani, N., Cavoski, I., Angioni, A. (2012). Nematicidal activity of (*E*,*E*)-2,4-decadienal and (*E*)-2--decenal from *Ailanthus altissima* against *Meloidogyne javanica*. *Journal of Agricultural and Food Chemistry*, *60*(4), 1146–1151.
10. Chamcheu, J.C., Rady, I., Chamcheu, R.N., Siddique, A.B., Bloch, M.B., Mbeumi, S.B., Babatunde, A.S., Uddin, M.B., Noubissi, F.K., Jurutka, P.W., Liu, Y.Y., Spiegelman, V.S., Whitfield, G.K., El Sayed,K.A. (2018). Graviola (*Annona muricata*) exerts anti-proliferative, anti clonogenic and pro-apoptotic effects in human non melanoma skin cancer UW-BCC1 and A431 cells *in vitro*: Involvement of hedgehog signaling. *International Journal of Molecular Sciences*, *19*(6), art. no. 1791.
11. Chan, W.K. Tan, L.T., Chan, K.G., Lee, L.H., Goh, B.H. (2016). Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules*, *21*(5), art. no. 529.
12. Cheng, M.C., Lin, L.Y., Yu, T.H., Peng, R.Y. (2008). Hypolipidemic and antioxidant activity of mountain celery (*Cryptotaenia japonica* Hassk) seed essential oils. *Journal of Agricultural and Food Chemistry*, *56*(11), 3997–4003.
13. Coria-Téllez, A., Montalvo-Gonzalez, E., Yahia, E., Obledo--Vázquez, E. (2018). *Annona muricata*: a comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. *Arabian Journal of Chemistry*, *11*(5), 662–691.
14. da Silva, L.M.R., de Figueiredo, E.A.T., Ricardo, N.M.P.S., Vieira, I.G.P., de Figueiredo, R.W., Brasil, I.M., Gomes, C.L. (2014). Quantification of bioactive compounds in pulps and by-products of tropical fruits from Brazil. *Food Chemistry*, *143*, 398–404.
15. da Silva, A.C., Jorge, N. (2017). Bioactive compounds of oils extracted from fruits seeds obtained from agroindustrial waste. *European Journal of Lipid Science and Technology*, *119*(4), art. no. 1600024.
16. Eromosele, C.O., Eromosele, I.C. (2002). Fatty acid compositions of seed oils of *Haematostaphis barteri* and *Ximenia Americana*. *Bioresource Technology, 82*(3), 303**–**304.
17. George, V.C., Kumar, D.R.N., Suresh, P.K., Kumar, R.A. (2015). Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extracts. *Journal of Food Science and Technology*, *52*(4), 2328–2335.
18. Huang, W.Y., Cai, Y.Z., Corke, H., Su, M. (2010). Survey of antioxidant capacity and nutritional quality of selected edible and medicinal fruit plants in Hong Kong. *Journal of Food Composition and Analysis*, *23*(6), SI, 510–517.
19. Jiménez, V.M., Gruschwitz, M., Schweiggert, R.M., Carle, R., Esquivel, P. (2014). Identification of phenolic compounds in soursop (*Annona muricata*) pulp by high-performance liquid chromatography with diode array and electrospray ionization mass spectrometric detection. *Food Research International*, *65*, SI, 42–46.
20. Kosińska, A., Karamać, M., Estrella, I., Hernandez, T., Bartolome, B., Dykes, G.A. (2012). Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *Journal of Agricultural and Food Chemistry*, *60*(18), 4613–4619.
21. Kuchtová, V. Kohajdová, Z., Karovičová, J., Lauková, M. (2018). Physical, textural and sensory properties of cookies incorporated with grape skin and seed preparations. *Polish Journal of Food and Nutrition Sciences*, *68*(4), 309–317.
22. Loizzo, M.R., Tundis, R., Bonesi, M., Menichini, F., Mastellone, V., Avallone, L., Menichini, F. (2012). Radical scavenging, antioxidant and metal chelating activities of *Annona cherimola* Mill. (cherimoya) peel and pulp in relation to their total phenolic and total flavonoid contents. *Journal of Food Composition and Analysis, 25*(2), 179–184.
23. Marques, V., Farah, A. (2009). Chlorogenic acids and related compounds in medicinal plants and infusions. *Food Chemistry*, *113*(4), 1370–1376.
24. Miller, H.E. (1971). A simplified method for the evaluation of antioxidants. *Journal of the American Oil Chemists' Society, 48*, 91–91.
25. Moghadamtousi, S.Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H.M., Kadir, H.A. (2015). *Annona muricata* (*Annonaceae*): a review of its traditional uses, isolated acetogenins and biological activities. *International Journal* of *Molecular Sciences, 16*(7), 15625–15658.
26. Moon, D.O., Kim, M.O., Choi, Y.H., Kim, G.Y. (2008). Beta--sitosterol induces G2/M arrest, endoreduplication, and apoptosis through the Bcl-2 and PI3K/Akt signaling pathways. *Cancer Letters, 264*(2), 181–191.
27. Nam, J.S., Park, S.Y., Jang, H.L., Rhee, Y.H. (2017). Phenolic compounds in different parts of young *Annona muricata* cultivated in Korea and their antioxidant activity. *Applied Biological Chemistry*, *60*(5), 535–543.
28. Nawwar, M., Ayoub, N., Hussein, S., Hashim, A., El-Sharawy, R., Wende, K., Harms, M., Lindequist, U. (2012). A flavonol triglycoside and investigation of the antioxidant and cell stimulating activities of *Annona muricata* Linn. *Archives of Pharmacal Research*, *35*(5), 761–767.
29. Orak, H.H., Karamać, M., Amarowicz, R., Orak, A., Penkacik, K. (2019). Genotype-related differences in the phenolic compound profile and antioxidant activity of extracts from olive (*Olea europaea* L.) leaves. *Molecules*, *24*(6), art. no. 1130.
30. Oreopoulou, W., Tzia, C. (2007). Utilization of plant by-products for the recovery of proteins, dietary fibers, antioxidants, and colorants. In Oreopoulou, V. and Russ, W. (Eds.) *Utilization of By-Products and Treatment of Waste in the Food Industry*, Springer Science and Business Media, New York, USA, pp. 209–232.
31. Pinto, L.C., de Souza, C.O., de Souza, S.A., da Silva, H.B., da Silva, R.R., Cerqueira-Lima, A.T., Teixeira, T.O., da Silva, T.M.S., Medeiros, K.C.P., Bittencourt, M., Brandão, H.R., Druzian, J.I., Conceição, A.S., Lopes, M.V., Figueiredo, C.A. (2018). Potential of *Annona muricata*L. seed oil: phytochemical and nutritional characterization associated with non-toxicity. *Grasas Aceites, 69* (1), art. no. e234.
32. Re, R., Pellegrini, N., Protrggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9–10), 1231–1237.
33. Sagar, N.A., Pareek, S., Sharma, S., Yahia, E.M., Lobo, M.G. (2018). Fruit and vegetable waste: Bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 512–531.
34. Zhishen, J., Mengcheng, T., Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*(4), 555–559.

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