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Original Article

Sour-Cherry Seed Polyphenol Contents, Antioxidant Activity and Nutritional Components as a Potential Bioactive Source

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ABSTRACT

Background and Objectives: Sour-cherry seed is one of the waste products of the sour-cherry jam and juice processing. Sour cherries include two valuable wastes, including sour-cherry kernels and seed shells. The two sections have been reviewed and assessed.

Materials and Methods: In this study, total phenolic compounds, DPPH radical scavenging activity, cellulose, hemicellulose, lignin, BET surface area and density of sour-cherry seed powder were assessed. Cytotoxicity of the methanolic extract of sour-cherry shell was investigated on breast cancer cell lines (MCF-7).

Results: Sour-cherry seed included high quantities of total phenols (27.02 mg GAE/g db). The high-performance liquid chromatography analysis of phenols identified chlorogenic acid 1887.50 (µg/mg), 3,4-dihydroxybenzoic acid 262.30 (µg/mg), quercetin 13.50 (µg/mg) and rutin 58.45 (µg/mg). Results reported 1.490, 36.65, 17.68 and 37.2% db of hemicellulose, cellulose and lignin content, respectively. Sour-cherry and walnut shell methanolic extracts significantly decreased MCF-7 cell growth (p <0.05) in a dose-dependent manner. The methanol extracts of walnut shells and sour-cherry seeds showed cytotoxic activities against MCF-7 cells with IC_{50} values of 0.47 and 0.97 mg/mL respectively. Kernels included 17% oil and 28.4% protein. Sour-cherry kernel oil included palmitic acid (5.93), stearic acid (3.3), arachidic acid (1.26), oleic acid (45.03), linoleic acid (40.61) and linolenic acid (3.87). Quantity of total phenolic compounds was reported as 6.41 mg gallic acid per gram.

Conclusions: In conclusion, sour-cherry seed showed good physical characteristics, including potentials to be used in sports drinks, health supplements, pharmaceutical carriers and biosorbents. Preliminary data on characteristics of the sour-cherry seed can provide useful information for potential uses in natural supplements as well as healthy foods.

Keywords: Sour-cherry seed, Phenolic compound, DPPH, Physical characteristics, Bioactive

Introduction

Sour cherry (*Prunus cerasus* L.) is one of the major fruit species in the Caucasus region, from Anatolia in the west to the north of Iran (1). Iran is ranked six in sour-cherry production in the world (109,707 Mt in 2018). Sour cherry is a fruit that produces huge waste after processing. Sour-cherry byproducts include pomace (skin and flesh) and seeds (pit and stone) which are wasted after juicing and individual quick freezing (IQF) processing (2). The biggest problem for food industries is waste production. Use of these wastes and production of value-added products are the concerns of food industries (2). High-value nutritional and functional compounds can be reused by extracting food wastes (3). Sour-cherry seeds include two major parts of shell (75–80%) and kernel (20–25%) (4). Use of shells includes heavy metal carriers and activated carbon. Altun (5) suggested use of sour-cherry kernel shells (SCS) for the removal of heavy metals as a low-cost waste from the beverage industries. Moreover, kernel shells can be used as environmentally-friendly adsorbents for heavy-metal removal. Walnut shell flour in small particle sizes (< 40 µ) can be used in cosmetic facial powder as body oil and moisture absorber (6). Shahidi *et al*. (7) reported that various hazelnut byproducts included excellent, valuable natural ingredients. Phenolic compounds are essential aspects of nutrients in plants. These compounds include potential agents for preventing various diseases (8).

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Cancer is a disease caused by abnormal proliferation of cells in the body. Human body consists of millions of cells. They are grouped together to form various tissues and organs. Naturally, cells are commanded by their genes. Sometimes, these commands are in vague and confused cells. Cell behaves abnormally and a group of abnormal cells can enter the bloodstream or immune system and/or form malignant tumors (cancers) (9). In cancer cells, cell cycle is not normally controlled and cells do not respond naturally to the body control mechanisms. Proteins involved in the signaling pathways that affect the cell cycle are altered and cell-cycle disruption is seen, with cells behaving differently and requiring various therapies (10). Breast cancer is one of the most common cancers in women in developing countries (11). It is the third leading cause of cancer death in Iran after cardiovascular diseases (CVDs) and accidents (10). The MCF-7 cells are epithelial-like cells derived from the breast cancer. This cell line includes general characteristics of the mammalian epithelia such as the ability to process estradiol through cytoplasmic estrogen receptors (12).

Despite much research on various cancers and their treatments, the disease is still one of the greatest health problems in human societies. Due to the fact that chemical drugs used in the treatment of cancers may cause drug resistance as well as other side effects, study on factors of natural origins such as compounds derived from plants with less harmful effects is one of the most important solutions for these problems. The major goals of the research are linked to the treatment of cancers (13). It has been reported that consumption of food products includes effective roles in preventing cancers due to the antioxidant characteristics of the products, especially their phenolic compound contents (14). Investigation of the relationships between the phenolic acids and their subclasses and the breast cancer showed that the biochemical compounds could decrease the risk of developing this cancer (15). These compounds are classified into various forms, including simple phenol, phenyl acetic acid, hydroxyl benzoic acid, hydroxyl cinnamic acid and other phenylpropanoids (7). Phenolic compounds and other bioactive constituents in fruit wastes can include multifunctional benefits such as health-promoting effects and replacing synthetic additives (16). Therefore, the major aims of this study were to assess sour-cherry seed chemical and physical characteristics, polyphenol content, antioxidant activities and nutritional components as potential bioactive sources. In addition, cytotoxicity of the methanolic extracts of sour-cherry and walnut shell on MCF-7 cancer cell line was investigated.

Materials and Methods

Materials

Folin-Ciocalteu, sodium carbonate, gallic acid and methanol with a purity of 100% were purchased from Merck, Germany. Sour-cherry waste shells were supplied by Shana Food, Karaj, Iran.

Sample preparation

Sour-cherry kernels were dried in shade at ambient temperature. After breaking the seeds, separated shells were crushed and powdered using blender and sifted using 40-mesh sieve.

Extraction of phenolic compounds

Phenolic compounds were extracted using shaking method (17). Briefly, 1.5 g of the sour-cherry powder were mixed with 30 mL methanol solvent at 25 ˚C. Mixture was transferred into an incubator shaker (IKA Labortechnik KS501, Germany) and incubated for 15 min at 150 rpm in dark. Sample was centrifuged (2K15, Sigma, Germany) at 3000 rpm for 15 min and the supernatant was separated. After the removal of the solvent using rotary evaporator (BUCHI Rotavapor R-114, Germany), the phenolic compounds were analyzed.

Extraction of sour-cherry kernel oil

Sour-cherry kernels were dried at room temperature in shade. After separating the shells, kernels were roasted at 160 °C for 20 min using oven (WISEVEN, Germany). These were crushed using mill (Spain, Moulinex) and prepared for solvent extraction based on a method by Yilmaz and Gokman (18). Then, 20 g of the milled sample were extracted at 60 °C for 24 h using Soxhlet oil. The solvent was removed using rotary evaporator (BUCHI Rotavapor R-114, Germany) and the resulting oils were sealed in bottles and stored at 4 °C until use.

Quantification of total phenolic compounds

Total phenolic content was assessed using Folin-Ciocalteu method (19). Gallic acid was used as standard and samples were read at 765 nm in triplicate using spectrophotometer. The total phenolic content was expressed in milligrams equivalents of gallic acid (GAE) per gram of each fraction. Then, 0.5 mL of the extracted solution was mixed with 2.5 mL of the Folin-Ciocalteu reagent (10-fold dilution). After 3 min of mixing, 2 mL of 7.5% sodium carbonate solution were added to the mixture. The reaction was heated at 45 ˚C for 15 min. Then, absorbance was read at 765 nm using blank of methanol.

High-performance liquid chromatography analysis of phenolic compounds

Waters liquid chromatography apparatus was used for the high-performance liquid chromatography (HPLC) analysis. The apparatus included a separation module (Waters 2695, USA) and a PDA detector (Waters 996, USA). Data acquisition and integration were carried out using Millennium 32 Software. The HPLC analysis of phenolic compounds was carried out based on a method by Tarnawski et al. (20) with slight modifications. Flow rate was 1 mL min^{-}

[1] and temperature was 25 °C. The mobile-phase composition included methanol and water. Separation was carried out on Eurospher 100-5 C_{18} (Knauer, Shimadzu) Reversed-phase Column (15 cm × 4.6 mm, 3.5 µm). Peaks were monitored at 195–400 nm and the injection volume was 20 µL. Identification of phenolic acids was based on retention times in comparison with standards. Concentrations of the compounds were calculated from the peak areas based on calibration curves. Quantity of each phenolic acid was expressed as µg/mg sample.

DPPH radical scavenging activity

Assessment of the free radical scavenging of sour-cherry shell extracts was carried out using DPPH method (19). Briefly, DPPH (1,1-diphenyl-2-picrylhydrazyl) solution (200 µM) was mixed with various concentrations of the samples. Absorbance was recorded at 517 nm after 30 min of storage at room temperature in dark. The DPPH quenching ability was expressed as IC50 (the extract concentration needed to inhibit 50% of the DPPH in the assay media. The IC50 values were achieved based on the linear regression equation for sour-cherry shells. Butylated hydroxytoluene (BHT) was used as standard and its IC50 value was reported as 11.64 µg/ml. Data at each concentration of the standards and samples were achieved from four replicates.

Cellulose, hemicellulose and lignin analysis

Cellulose, hemicellulose and lignin contents were assessed using the method of Li et al. (21). The sour-cherry shells (G0, g) were washed with a mixture of benzene/ethanol (2:1 in volume) at a constant temperature for 3 h. After air- drying, residue was dried at 105–110 °C to a constant weight using oven. Then, residues were cooled down to room temperature using desiccator and weighed (G1, g). The extractive wt. % was calculated using Eq. 1.

$$W_1 \text{ (wt. \%, d)} = \frac{G0 - G1}{G0} \times 100\% \quad (1)$$

Where, W_1 was the extractive (%).

Hemicellulose analysis

The G1 residue from the extractive analysis was transferred into a flask and mixed with 150 ml of NaOH (20 g/l). The mixture was boiled for 3.5 h with recycled distilled water (DW). The residue was filtered and washed until removal of all Na^+ and then dried to a constant weight. This was cooled down to room temperature in a desiccator and weighed (G2, g). The hemicellulose wt. % was calculated using Eq. 2.

$$W_2 \text{ (wt. \%, d)} = \frac{G1 - G2}{G0} \times 100\% \quad (2)$$

Where, W_2 was the hemicellulose (%).

Lignin analysis

One gram of the residue after the extractive analysis was transferred into a weighed flask and died to a constant weight. Sample was cooled down in a desiccator and weighed (G3, g). Slowly, 30 ml of sulphuric acid (72%) were poured into the sample. Mixture was stored at 8–15 °C for 24 h; then, transferred into a flask and diluted with 300 ml of DW. Sample was boiled for 1 h with recycled DW. After cooling down and filtration, residue was washed until removal of sulfate ions in the filtrate (detected by 10% barium chloride solution). Then, residue was dried to a constant weight, cooled down to room temperature in a desiccator and weighed (G4, g). The lignin wt. % was calculated using Eq. 3.

$$W_3 \text{ (wt. \%, d)} = \frac{G4(1 - W1)}{G3} \times 100\% \quad (3)$$

Where, W_3 was the lignin (%).

Cellulose analysis

The cellulose wt. % was calculated using Eq. 4.

$$W_4 \text{ (wt. \%, d)} = 100 - (W_1 + W_2 + W_3) \quad (4)$$

Where, W_4 was the cellulose (%).

Sour-cherry kernel protein content

Sour-cherry kernel protein was assessed before oiling to better identify compositions of the sour-cherry kernels using Kjeldahl method (26).

Fourier-transform infrared spectrometry

Sour-cherry and walnut shell samples were assessed using Fourier-transform infrared spectrometer (Tenosor 27, Bruker, Germany). Fourier-transform infrared (FTIR) spectra provided information on the characteristics of functional groups on the surface of raw sour-cherry shells. Kirby-Bauer disk method was used for the sample and the wavelength was set at 400–4000 cm^{-1} at a resolution of 4 cm^{-1}. Detection was based on the peak values in the region of infrared spectra, described previously by Li et al. (21).

Brunauer-Emmett-Teller surface area analysis and density assessment

Specific surface areas of the sour-cherry shells were assessed using Brunauer-Emmett-Teller (BET) instrument (Model Belprep II, Japan). Characterization of the specific surface areas, pore volumes and pore diameters of sour-cherry shells was carried out using BET method (22). Briefly, 10 points with the P/P0 ratio were used as standard measurement points. Technically, 0.2145 g of the sample was weighed and analyzed with a standard volume of 0.8962 cm^3, dead volume of 15.336 cm^3 and saturated vapor pressure of 89.202 Kpa. Specific gravity was assessed based on D854-14 standard.

Sour-cherry kernel oil fatty-acid profiles

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This was carried out based on the method of Asadi et al. (24). Briefly, 200 mg of the oil were mixed with 10 ml of hexane and 20 ml of 1% by weight volumetric methanol, boiled for 10 min and centrifuged at 2000 rpm for 2 min (Sigma-Aldrich, Germany). Then, 1 µl of the sample was injected into gas chromatographer with a ratio of 1:40. The fatty acid (FA) composition was assessed using Varian CP-3800 Gas Chromatography System with a capillary column (Bpx 70) (SGE, Melbourne, Australia). Temperatures of the detector and injector included 250 and 230 °C, respectively. The column temperature was initially set at 60 °C for 60 min and then increased to 200 °C at a rate of 4 °C/min. Velocity of the carrier gas (helium) was 1.5 ml per min. After injecting the sample into the gas chromatographer, a curve was plotted and the inhibition time of each FA was compared with that of the curve of standard FA and its inhibition time. Thus, type and quantity of FAs in the sample were assessed and expressed in percentage. Acidity of the oil, peroxide value of the oil and refractive index were assessed based on the method of Fernandes et al. (25).

Cytotoxicity assessment of the methanolic extracts from sour-cherry and walnut shells

The MCF-7 cell line was provided by the National Cell Bank of Iran (Pastor Institute, Tehran, Iran) and cultured in RPMI-1640 media supplemented with 10% FBS (Gibco, USA), 100 mg/mL streptomycin and 100 U/mL penicillin G and incubated at 37 °C in humidified air containing 5% CO_2. Cytotoxic activity of the sour-cherry and walnut shell extracts was assessed using MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma-Aldrich, USA) assay (23). After trypsinization and separation of the cells from the surface of the 25T flask (SPL, Korea), cells were harvested, counted and diluted to 1×10^4 cell/mL. Then, 190 µL of the cell suspension were seeded in 96-well culture plates and incubated overnight at 37 °C in a humidified air atmosphere with 5% CO_2. After incubation, 10 µL of various concentrations (0.125, 0.250, 0.5 and 1 mg/mL) of the methanolic extracts and DMSO solvent (1%) as negative control was added to the wells and incubated for 48 h. It is noteworthy that the effects of each concentration of the extract on MCF-7 cell line were investigated in three independent experiments in triplicate. After incubation, media were replaced with 200 µL of phenol red-free media containing MTT (1 mg/mL) and incubated for 4 h. Then, supernatants were discarded and 100 µL of DMSO were added to each well and absorbance values were measured at 492 nm using multi-well plate reader (Gen5, Epoch, BioTek, USA). Inhibition proportion and concentration causing 50% inhibition in cell growth (IC_{50}) were calculated via nonlinear regression analysis and expressed in mean ±SD (standard deviation). To investigate cytotoxicity of the methanolic extracts of sour-cherry and walnut shells, various concentrations of the extracts were prepared and cytotoxic effects of each concentration were assessed on MCF-7 cancer cell line.

Statistical analysis

Results were reported as the mean of three independent assessments. Principal component analysis was carried out using Minitab Software v.16.

Results

Phenolic compounds

Phenolic compound content of the sour-cherry shells was 27.02 mg GAE/g dry weight. Figure 1 shows compounds of the sour-cherry shells using HPLC analysis. These compounds included 3,4-dihydroxybenzoic acid, chlorogenic acid, quercetin and rutin at concentrations of 262.30, 1887.50, 13.50 and 58.45 (µg/mg sample), respectively (Table 2). The SCK, SCP and SCS included two similar compounds of chlorogenic acid and quercetin (Table 1). Rutin and 3,4-dihydroxybenzoic acid were detected in SCS, which have not been detected in other parts of the sour cherries. These data revealed that proportions of the phenolic subclasses were similar to SCK, SCP and SCS, although quantities were significantly different.

The HPLC analysis of phenolic acids in SCS demonstrated that the highest value belonged to chlorogenic acid and the lowest value belonged to quercetin (Table 2).

Table 2. Major phenolic compounds in the sour-cherry shells

Name	*Retention time (minutes)*	*Quantity (µg/mg sample)*
Chlorogenic acid	15.877	1887.50±0.02
3,4-dihydroxybenzoic acid	11.065	262.30±0.07
Quercetin	36.006	13.50±0.03
Rutin	29.053	58.45±0.09

The data mean 3 replicates (standard deviation ± mean).

DPPH radical scavenging activity

Free-radical scavenging activities of the sour-cherry shells were assessed. Linear regression equation for the sour-cherry shell was $y = 0.0423x + 3.8989$ ($R^2 = 0.9945$). The IC50 value was concentration of the sample needed to inhibit 50% of the radicals. The IC50 in sour-cherry shells was 1089.861 µg/ml. Based on a study by Marjoni et al. (33), this quantity belonged to the fifth group with inactive intensity, in contrast to a review by Molyneux (34). This revealed that an IC50 of 200–1000 µg/ml included weak intensity, but included the potential of antioxidant.

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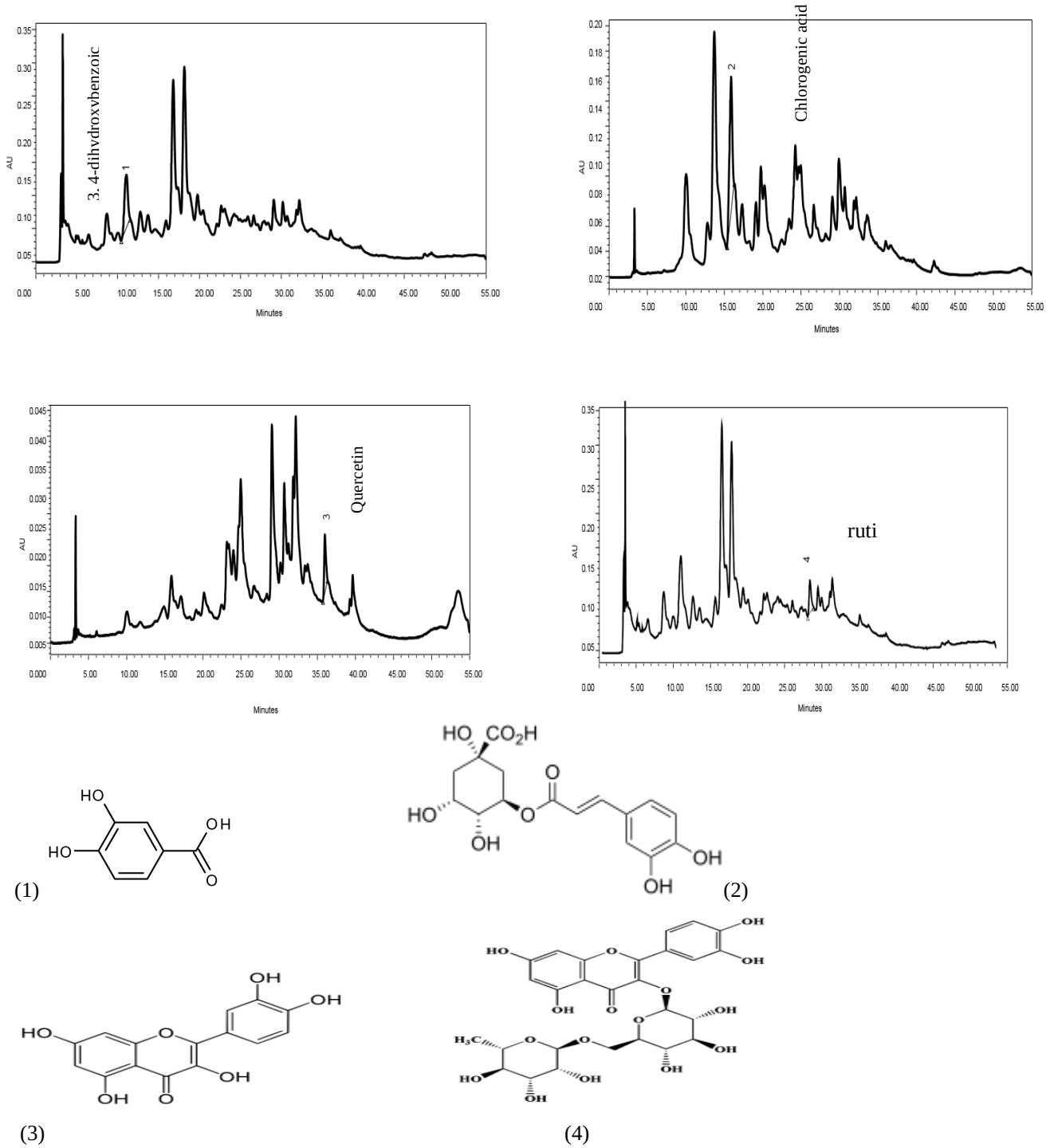

Figure 1. The HPLC chromatograms with UV detection. (1) at 260.33 nm, showing composition of 3,4-dihydroxybenzoic acid; (2) at 326.7 nm, showing composition of chlorogenic acid; (3) at 255.6 nm, showing composition of quercetin; and (4) at 256.8 nm, showing composition of rutin

Chemical composition

Based on Table 3, cellulose and hemicellulose contents in the sour-cherry shells were lower than those in walnut and almond shells. Lignin content of the sour-cherry shells was lower than that in walnut shells and higher than that in almond shells. The extract content indicated presence of terpenes, phenols, hydrocarbons and ester compounds.

Table 3. Comparison of cellulose, hemicellulose and lignin contents in three shell wastes (wt. %)

	Cellulose	Hemicellulose	Lignin
Sour-cherry shell	27.85±0.55	25.83±0.42	39.73±0.71
Almond shell*	38.47	28.82	29.45
Walnut shell*	36.38	27.85	43.70

The data of sour-cherry shell were measured by authors and the rest of the measurements are derived from* Li et al. (21).

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Infrared spectrum

The FTIR spectra of sour-cherry and walnut shells are shown in Figure 2. These showed similar compounds in the spectra. Peak range and sharpness of the waves demonstrated differences in the number of compounds. Peak of 890 cm^{-1} was a characteristic of cellulose. This peak in the walnut shells was larger than that in sour-cherry shells. Peak of 1730 cm^{-1} showed that the content of hemicellulose was higher in walnut. Moreover, peak of 1455 cm^{-1} belonged to lignin. In general, peaks from the walnut shells were sharper and stronger than those from the sour-cherry shells.

Brunauer-Emmett-Teller surface area analysis and density assessment

Figure 3 demonstrates a typical Type III based on International Union of Pure and Applied Chemistry (IUPAC) classification. Isotherm curves of the sour-cherry shells were convex, illustrating weak interactions between the adsorption and desorption. Result of total pore volume, average pore diameter and density for sour cherry are summarized in Table 4. An average pore diameter of 6.25 nm was recorded, showing a mesoporous structure.

Table 4. Physical characteristics of the sour-cherry shells

Total pore volume (p/p_0=0.990)	0.0035547 (cm^3 g^{-1})
Average pore diameter	6.2532 (nm)
Specific gravity	1.22 g/cm^3

Specific gravity value of the sour-cherry shells was 1.22 g/cm^3, while the specific gravity values achieved in coconut, groundnut and palm kernel shells were 1.42, 1.48 and 1.61 g/cm^3, respectively.

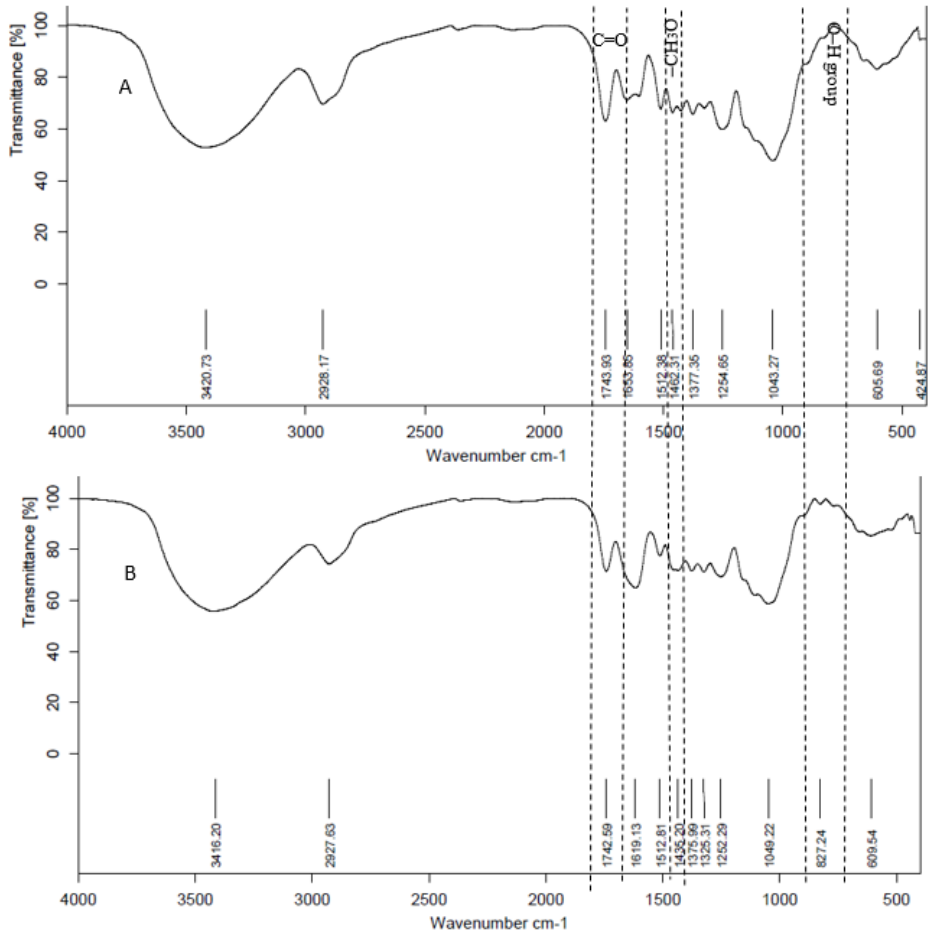

Figure 2. Fourier-transform infrared (FTIR) spectra. Walnut shells (A) and sour-cherry shells (B)

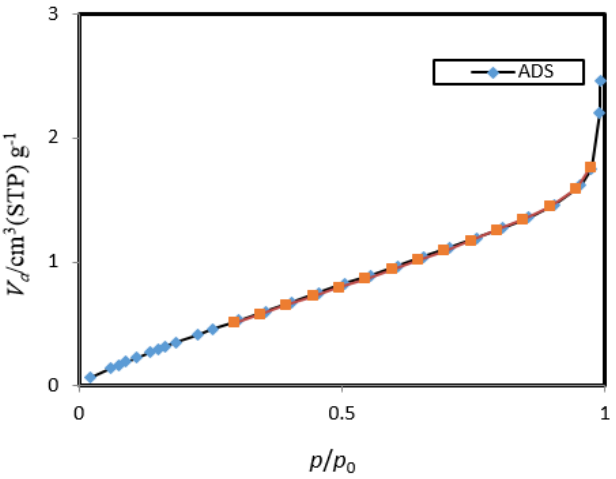

Figure 3. Adsorption-desorption isotherm curves of the sour-cherry shells. ADS, adsorption; DES, desorption

Cytotoxicity assessment of the methanolic extracts from sour-cherry and walnut shells

Figure 4 shows cytotoxicity of the methanolic extracts from sour-cherry and walnut shells. Result demonstrated that sour-cherry and walnut shell extracts at 0.125–1 mg/ml concentrations included inhibitory effects on breast cancer cell growth. Methanolic extract of the walnut shells showed a higher cytotoxic activity against MCF-7 with an IC_{50} of 0.47 mg/mL, compared to that methanolic extract of the sour-cherry shells with IC_{50} of 0.97 mg/mL did.

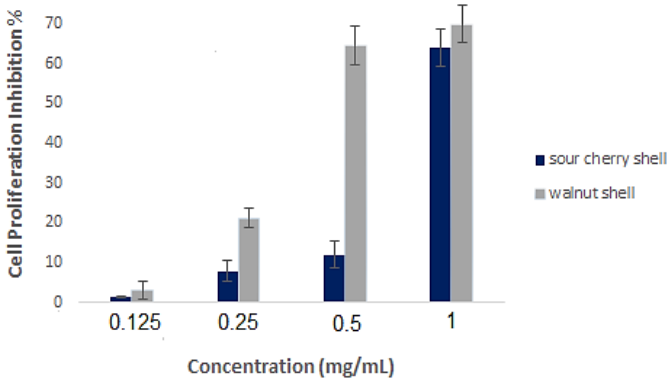

Figure 4. Effects of various concentrations of sour-cherry and walnut shell extracts on growth inhibition of MCF-7 cells (results are reported as mean ±SD)

Fatty acid profiles of the sour-cherry kernel oil

Saturated fatty acids (SFAs) in sour-cherry kernel oil included arachidonic acid, stearic acid and palmitic acid, which consisted of 10.49% of the total SFAs. The lowest quantity of SFA was linked to arachidonic acid. The predominant FA in sour-cherry kernels were unsaturated fatty acids (UFAs), including oleic acid, linoleic acid and linolenic acid (Table 5).

Table 5. Fatty acid profile of the sour-cherry kernel oil

Fatty acid	Quantity (%)
Oleic acid	45.03±0.06
Linoleic acid	40.61±0.07
Linolenic acid	3.87±0.06
Arachidic acid	1.26±0.37
Palmitic acid	5.93±0.04
Stearic acid	3.3±0.05

The quantity of fatty acids mean values ± standard deviations in cherry kernel oil is expressed.

Peroxide value, acidity and refractive index of sour-cherry oil are shown in Table 6.

Table 6. Peroxide value, acidity and refractive index of the sour-cherry kernel oil

Peroxide value (meq O_2/Kg)	1.00±0.06
Acidity (mg KOH/g)	0.295±0.07
Refractive index	1.447±0.06

Values are expressed mean values ± standard

Sour-cherry kernel protein

Protein content of the sour-cherry kernel was 28.4%.

Discussion

Phenolic compounds are produced by the secondary metabolism of plants. Food processing produces a large quantity of these compounds (27), which are known to include health benefits in cancer, CVD and diabetes preventions (7). Results of this study showed that the phenolic content of sour-cherry shells was higher than that of walnut shells (25.6 mg GAE/g) (17) and lower than that of almond shells (78.2 6 mg GAE/g dry weight) (28). Moreover, phenolic compounds in pecan shells included 65.3–92.5 mg GAE/g shell (29). Chlorogenic acid is a strong antioxidant substance with a wide range of health advantages. It can play critical roles in glucose and lipid metabolism regulations and associated disorders such as diabetes, CVDs, obesity, cancers and hepatic steatosis (31). De la Rosa et al. (29) identified gallic and ellagic acid as major polyphenols in pecan shells. Results of this study suggested that the phenolic compounds extracted from sour-cherry seeds might be used in the production of dietary supplements and as ingredients in healthy drink formulation for athletes.

Technically, DPPH radical scavenging is used to assess overall antioxidant capacity of the sample (32). Studies have been carried out on the radical inhibition by various chemical concentrations. Furthermore, 0.05 mg/ml of Mexican pecan shells showed a 50% inhibition rate. Proportion of inhibition at a concentration of 0.1 (mg/ml) of the sour-cherry shells included 20.79%, indicating that the Mexican pecan shells included better inhibitory effects than that the sour-cherry shells did. At this concentration, proportion of inhibition in walnut shells included 7.19% (35), indicating that the sour-cherry shells included higher

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inhibitory effects than those the walnut shells did. Therefore, it can be concluded that by increasing concentrations of the phenolic compounds from cherry kernels, the inhibition rate increases. This could be due to the increased effects of the active ingredients as well as increased contents of their total phenolic compounds. De la Rosa et al. (29) showed that pecan kernel and shell extracts were highly effective DPPH scavengers; from which, the latter was almost three times stronger than the former.

Based on Queiros et al. (36) study on almond shells, the extract content included 5.7%. This in the sour-cherry shells was 6.59%, which showed that the sour-cherry shells included more terpenes, phenols, hydrocarbons and ester compounds than those the almond shells did. Cellulose, hemicellulose and lignin include great potentials of use in various industries. In one study, dialysis-free extraction and characterization of cellulose crystals from almonds showed that almond shells could be used as cellulose sources (37). Kabbashi et al. (38) reported that high lignin contents led to better carbon activation. Based on the data achieved in this study, further extensive studies on the use of cellulose, hemicellulose and lignin from sour-cherry shells for the production of activated carbons, diet foods and additives are necessary.

The BET analysis allows for a precise specific surface area assessment of materials through nitrogen multilayer adsorption measured as a function of relative pressure using fully automated analyzer. The technique includes external area and pore area assessments to assess the total specific surface area in $cm^3\ g^{-1}$, demonstrating that shell powder includes the potential to be used in cosmetics, pharmaceutical carriers and biosorbents (39). Pore diameters in coconut, groundnut and palm kernel shells included 2.840, 2.920 and 3 nm, respectively (40). A high BET level indicates the presence of more adsorption sites. Therefore, it can be used as a pollutant adsorbent. The BET surface area value was 2.2739 (m^2/g) in the sour-cherry shells, which was less than that in the coconut, groundnut and palm kernel shells. Boadu et al. (40) reported a specific gravity of 0.8–2.1 for the activated carbons in material safety data sheets (MSDS). Further studies can be carried out on the use of sour-cherry shells as activated carbons.

Nowadays, more than 60% of anticancer compounds are originated from plant, marine and microbial sources. Secondary metabolites in plants include several biological effects, including anti-inflammatory, anticancer, analgesic and cardiovascular effects (40). As shown in Figure 4, sour-cherry and walnut shell extracts at concentrations of 0.125–1 mg/ml included inhibitory effects on breast cancer cell growth. The methanol extract of walnut shells showed a higher cytotoxic activity against MCF-7 with IC_{50} concentration of 0.47 mg/mL, compared to that the sour-cherry shells with IC_{50} of 0.97 mg/mL did. With increasing concentration of the extracts from walnut shells and sour cherries, rates of the growth inhibition decreased. These possibly are linked to the effects of phenolic compounds in the shells of sour cherry and walnut. Results of this study were similar to those of studies by Bak et al. (29), who showed that oil-free kernel extracts included anticancer characteristics. Polyphenols are an important group of phytochemicals that have been interested by the researchers because they have been shown to affect growth of cancer cells (40). Studies on relationships between the anticancer effects of phenolic compounds and their structures with functional groups have demonstrated that compounds with further hydroxyl groups include better anticancer performances. For example, a study by Lee et al. (41) to assess effectiveness of benzoic acid in inhibiting growth of cancer cells showed that cinnamic acids with unsaturated propionic acid side chains were better anticancer agents. Therefore, phenolic acids of benzoic and cinnamic acid derivatives with higher hydroxyl substitutions can be considered as potential candidates to prevent the proliferation of cancer cells (42). In this study, relationships were seen between the anticancer activity of phenolic compounds and the structure with functional groups such as aromatic rings and hydroxyl groups, including the highest effects at concentrations of 1 mg/ml.

Popa et al. (45) reported that sour-cherry kernel oil included small quantities of arachidic acid (0.9%) and myristic acid (0.5%) while predominant SFAs included palmitic acid (11%) and stearic acid (6.4%). Quantity of linoleic and linolenic acids (44.48%) were detected in sour-cherry kernel oil. Linoleic and linolenic UFAs, classified as W6 and W3 UFAs, are important parts of the human diets because their absence in diets includes adverse effects on human health such as occurrence of CVDs and skin lesions. Comparing sour-cherry kernel oil with sunflower oil, sour-cherry kernel oil includes higher oleic acid contents. Despite similarities between the sunflower oil and sour-cherry kernel oil, sour-cherry kernel oil includes acids. High free fats are not detected in sunflower oil [41]. Chandra and Nair (46) reported that oleic acid and linoleic acid contents in cherries as 63.5 and 31.5%, respectively, similar to those in safflower, olive and sunflower oils. In a study by Fernandez et al. (25) on tricolor oil of almond, hazelnut and walnut, the highest proportion of FAs belonged to oleic acid. Based on Table 5, sour-cherry kernel oil can be used in combination with other foods or nutrients containing W3, which can include health effects on chronic diseases. Sour-cherry kernel oil can be used in combination with olive or canola oil for oral consumption.

Peroxide values vary based on the position and number of bonds in the UFAs. Peroxide value of sour-cherry seed oil was 1 meq O_2/Kg. Peroxide value shows measure of the progress of oxidation reactions in oils and fats. Quality of

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sour-cherry kernel oil is naturally high. In this study, the average acidity of this oil was 0.295 mg KOH/g. Acidity in kernel oil of palm, mango 1.2 (47) and 1.5 mg KOH/g (48) were reported, respectively. Furthermore, this parameter demonstrates good quality and healthiness of the sour-cherry kernel oil. The refractive index of sour-cherry kernel oil was calculated as 1.44, which was expressed as 1.46 in grape kernel oil (29). Plant proteins play important roles in human nutrition, especially in developing countries where the average protein intake is less than the basic levels. Due to the lack of dietary proteins, plant proteins have been further popular as sources of novel proteins, which are used as functional foods and in dietary supplements (30). Sour-cherry kernel proteins are non-toxic plant proteins that are rich in essential amino acids (ESAs) such as glutamic acid, arginine, aspartic acid and serine (49). Sour-cherry kernel proteins can be suggested as ingredients in cookie formulations, foods containing cereals and beverages for athletes.

Conclusion

Natural originated materials such as compounds derived from fruit processing wastes and byproducts are mostly targeted in recent studies on functional and nutraceutical foods. In the present study, sour-cherry seed components were investigated. The phenolic compounds and antioxidant characteristics identified in the sour-cherry shells showed that this wastes could be used as good sources of health-promoting compounds. Methanolic extract of sour-cherry and walnut shells significantly inhibited MCF-7 cancer cell growth at concentrations of 0.125–1 mg/ml. Chemical composition of the sour-cherry kernel oil was suggested for possible use as a novel source of salad oil, frying oil and processed foods. High lignin content and specific gravity revealed that the sour-cherry shells could be used as agricultural wastes in production of activated carbon as well as using cellulose in production of healthy and cosmetic products with high cellulose contents. The shells include mechanical and physical characteristics and could be used in fibrous products, cosmetic products, fillers, medicinal and fragrance ingredient. Results of the present study showed that sour-cherry kernels included biological compounds from natural sources that could be used in healthy foods.

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Ethical statement

Ethics approval was not necessary for this study.

Financial disclosure

The authors declare no conflict of interest

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