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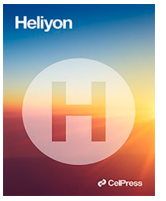

Research article

Phenolic components from carrot (*Daucus carota* L.) pomace: Optimizing the extraction and assessing its potential antioxidant and antimicrobial activities

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ABSTRACT

Carrot pomace is a significant agricultural byproduct. Obtained during carrot juice processing. This residue is an appropriate reservoir of constituents with bioactive properties that could be investigated in the development of food constituents and nutritional supplements and in improving the quality and safety of foods. For this purpose, the objective of the present investigation was to extract the polyphenols from carrot pomace utilizing maceration and ultrasound-based extraction (UAE) procedures and to evaluate the antioxidant properties of phenolic constituents. To maximize the extraction of carrot pomace, a response surface approach was used. The optimal mixture of extraction time (A, min), ultrasonication power (B, w), and solvent type (C, v/v) for the highest yield of carrot pomace was found using a three-variable composite rotatable design (CRD). In order to assess different functional groups, Fourier transform infrared spectroscopy was utilized to investigate the extract collected under optimal circumstances. The highest polyphenols (26.53 %) were extracted by ethanol 70 % at 10 min with a sonication power of 250 w. The optimized extract also exhibited significant antioxidant and antimicrobial functions. The total phenolic compounds and scavenging of the DPPH radical were 85 mg GAE/gr and EC_{50}: 55 ± 1 μg/mL, respectively. Together with *Staphylococcus aureus*, the highest zone of inhibition (12 mm) was identified. Our finding revealed that carrot pomace is an appropriate source of bioactive phenolic constituents, exhibiting antioxidant and antibacterial attributes, and could be applied as a natural preserver for promoting safety and quality properties in food products on an industrial scale.

1. Introduction

Numerous different bioactive substances may be found in fruits and vegetables, such as vitamins, dietary fiber, and minerals, that are essential for our well-being. Over recent years, the global production and consumption of carrots and their products have been progressively increasing. In 2015, carrot production was reported to be 40.2 million metric tons, which increased to 44.8 million metric tons in 2019 with a growth level of 10.3 % [1]. Carrot (*Daucus carota* L.) is a biennial edible root vegetable in the *Apiaceae*

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(previously *Umbelliferae*) family. This is the sixteenth largest family, including 455 genera and more than 3700 species. The vegetable possibly originated in ancient Persian and was initially grown for its leaves and seeds. Carrot pomace (carrot waste), is the key secondary product of the juicing process, and it is an excellent source of insoluble fiber-rich fraction (IFRF), phenolics (especially hydroxycinnamic acid derivatives), minerals (iron, potassium, zinc, manganese, etc.), proteins, and vitamins [2].

Recently, due to the beneficial effects of valuable bioactive substances from natural sources on human health, there has been increasing motivation for their recovery. Natural polyphenolic compounds have exhibited numerous biological functions, such as anti-inflammatory, antigenotoxic, pro-apoptotic, cardio-protective, anti-tumor, and antimicrobial properties, predominantly due to their antioxidant and antiradical properties [3,4]. Phenolic substances possess an aromatic ring that has a number of hydroxyl substitutes in their chemical structure. In terms of chemical structure, plant phenolic compounds may be simple; for example, benzoquinones (C_6) and hydroxybenzoic acids (C_6-C_1) may both be generated in a polymeric state as lignin (C_6-C_3)n and dense tannins (C_6-C_3-C_6)$_n$ and have complicated chemical structures [5,6].

Extraction is an appropriate method employed for gathering pure ingredients or constituents from a liquid or solid mixture. This procedure is the most imperative phase to isolate polyphenols from plant substances. The bioactive constituents can be extracted utilizing both non-conventional and conventional manners. Non-conventional (also called green extraction technology) supercritical fluid-, enzyme-assisted-, and ultrasound-assisted extractions, have lots of benefits, such as high yield, short time, low energy usage, and high-quality products [7–10].

Generally, there are numerous extraction actions such as maceration, pulsed electric field (PEF) assisted extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), high voltage electrical discharges (HVED) extraction, supercritical fluid extraction (SFE), pressurized fluid extraction (PFE), enzyme-assisted extraction (EAE), and high pressure-assisted extraction (HPAE) [4,5,8]. Among them, maceration is one of the widespread conventional solid-liquid extraction procedures that is frequently utilized for gaining different bioactive constituent extracts from plant substances [9,11,12]. Ultrasound-assisted extraction, also known as ultrasonic extraction (USE), has been considered a favorable and inventive procedure with numerous uses in the alimentary, pharmaceutical, chemistry, and cosmetic fields of the 21st century [13–15]. This study was intended to primarily optimize the extraction situations of polyphenols from carrot pomace and then to assay their potential antioxidant and antimicrobial properties.

2. Material and method

2.1. Chemicals

Methanol, ethanol, acetone, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), gallic acid, were obtained from Sigma Aldrich (the United States of America). Mueller–Hinton Broth (MHB) and Agar (MHA) were acquired from Merck (Germany). Chemicals and the other solvents utilized were of analytical or chromatographic grade.

2.2. Preparation of carrot pomace

Wet carrot pomaces were collected from local fruit juice shops in Mashhad and dehydrated using an oven at 45 °C. Following the drying process, the pomaces were milled by a laboratory mill. Dried pomace samples were sieved using a standard sieve (mesh, 500 μm). After that, to prevent negative effects, the specimens were sealed with a vacuum in polyethylene bags and maintained at −80 °C until the research was finished [1]. Ultrasound treatment and antioxidant and microbial assays were performed on the optimal sample.

2.3. Extraction procedures

2.3.1. Maceration

Generally, maceration is the simplest method of solid-liquid extraction and involves placing the plant substance in contact with an organic solvent, such as methanol, ethanol, acetone, etc., with or without stirring at ambient or long durations of high temperatures [16]. Carrot pomace powder (10 gr) was added with 20 mL water-methanol (v/v: 20:80), and the blend was stirred for 8 h with a shaker at a velocity of 200 rpm. Subsequent, the tube underwent centrifugation at a speed of 6000 revolutions per minute for a duration of 15 min at a temperature of 4 °C, and the upper layer was harvested as a methanolic extract to perform further analyses [17].

2.3.2. Ultrasound-assisted extraction (UAE)

Ultrasound consists of mechanical waves that have frequencies above the range of human hearing, namely between 20 and 1000 kHz. Ultrasound-assisted extraction was performed according to Bamba and colleagues, with some modifications [18]. Ten grams of carrot pomace powder were mixed with ethanol (100 mL, 80 % v/v) and was subjected to sonication in a bath of ultrasonic waves at a temperature of 10 °C for a duration of 15 min. Afterward, the sample underwent centrifugation at a speed of 8000 revolutions per minute for a duration of 10 min at a temperature of 4 °C, and the obtained extract was subjected to evaporation in a rotating evaporator at a temperature of 65 °C under a vacuum. Finally, the specimen was heated in a vacuum oven at a temperature of 45 °C till complete dryness was attained.

2.4. Experimental design

The impact of three variables, including extraction time (A, min), ultrasonication power (B, w), and type of solvent (C, v/v), on yield extraction was optimized via response surface methodology (RSM). In this research, the optimization of solvent type, extraction duration, and ultrasonication power was carried out using a composite rotatable design. Since usually the extraction process of these compounds is under the direct influence of various factors, these factors may have synergistic or antagonistic effects on each other. Therefore, to produce an extract of the greatest possible quality and efficiency, it is necessary to study the optimal values between different factors and determine the best situation.

2.5. Extraction yield (%)

For the assessment of extraction yield, the potential interaction between three factors (solvent type, extraction time, and ultrasonication power) was investigated.

2.6. Total phenolic content (TPC) assay

By utilizing the Folin-Ciocalteu test in a manner slightly different from that recommended by Safdar and colleagues, the total phenolic component of the carrot pomace extract was assessed [11]. In brief, 2.5 mL of 10 % Folin-Ciocalteu reagent solved in demineralized water was added to 0.5 mL of methanolic extract solution. The mixture was agitated using a vortex mixer and then allowed to rest in a light environment at room temperature for 5 min. Later, 2.5 mL of 7.5 % sodium carbonate solution was added. The blank sample contained 2.5 mL of Folin-Ciocalteu reagent, 2.5 mL of 7.5 % sodium carbonate, and 0.5 mL of methanol solvent. Subsequently, all tubes were incubated for 30 min at 25 °C for blue color formation. A UV–Vis spectrophotometer measured the mixtures' UV absorbance at 765 nm. In order to determine the TPC in the samples, the absorbance was compared with the gallic acid calibration curve. The final outcomes were presented as mg of gallic acid per gram of extract.

2.7. DPPH radical scavenging assay

The DPPH test was used to evaluate the antioxidant properties of carrot pomace extracts. Basically, the initial mixture was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. Methanol and DPPH solutions were mixed to create the working standard, and a spectrophotometer was utilized to get an absorbance of around 0.980 at 517 nm. One hundred mL of specimens with diverse levels, between 12.5 and 400 μg/mL, were merged with 3 mL of the DPPH mixture. For 15 min, the mixture was permitted to come to ambient temperature while being gently stirred and kept in a dark area. Methanol was considered a blank. The sample's absorbance was finally determined via a spectrophotometer at a wavelength of 517 nm [13]. Based on the DPPH radical percentage, the scavenging capability was evaluated in the manner described below:

$$\text{Radical-scavenging capability } (\%) = \left(\frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}}\right) \times 100$$

Subsequently, the acquired value for each concentration was graphed in order to determine the EC50 values (the concentration at which fifty percent of the free radical content of DPPH is decreased).

2.8. Reducing power assay

After incubation, the potency of the extract to convert the ferric-ferricyanide complex of Prussian blue to the ferrous-ferricyanide was assessed by measuring the absorbance at a wavelength of 700 nm. The extracted specimen was combined with 1 mL of purified water, 1 mL of 1 % potassium ferricyanide K_3 ($Fe(CN)_6$), and 1 mL of phosphate buffer (pH 7.0). After incubation of the blend at 50 °C for 30 min, underwent centrifugation at a speed of 1500 revolutions per minute for a duration of 15 min. Afterward, the mixture's absorbance was measured by spectrophotometer at 700 nm using an aliquot (2 mL) of the top layer, 0.4 mL of 0.1 % ferric chloride ($FeCl_3$) solution, and 2 mL of deionized water. The reducing power's absorbance at 700 nm was used as an indication. The reaction mixture's high absorbance revealed a greater reduction capacity [19].

2.9. Antimicrobial assay

To assess the suppression of microbial growth, two important foodborne pathogens, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923, were elected. The antibacterial assay was carried out using 300 μL of standardized inoculum suspension comprising approximately 10^7 CFU/mL of bacteria. To make standardized inoculums, bacteria were grown in broth media at 37 °C for 48 h. The antimicrobial characteristics were evaluated by measuring the bacteria's inhibitory zone with a disk diffusion (DD) susceptibility assay. Within all four corners of the Petri dish, a disc was added after drying. After incubation at 37 °C for 48 h, by considering the diameter of the inhibitory area (mm) on the surface of every plate, the antimicrobial property was determined [20].

2.9.1. Minimum inhibitory concentration (MIC) assay

The minimum inhibitory concentration values were measured for the bacterial strains susceptible to the extracts in the well diffusion assay. Diverse levels (200, 300, 400, 500, and 600 mg/mL) of the extract were prepared. An aliquot (100 μL) of the resuspended extract was poured into the first well of a 96-well sterile plate before being filled with Mueller-Hinton broth (100 μL). By adding MHB and the bacterial inoculate into consecutive wells, serial two-fold dilutions were performed. Then, the specimens were incubated at 35 °C for 24 h. The control sample was MHB without any extract. The lowest concentration that resulted in the preservation or diminish of inoculums' viability was considered the MIC [21].

2.9.2. Minimum bactericidal concentration (MBC) assay

The minimum bactericidal concentration was assigned by incorporating different concentrations of extracts (200, 300, 400, 500, and 600 mg/mL) in Mueller-Hinton broth for both bacterial species. After being incubated for 48 h at 35 °C, the tubes that had no discernible growth were subcultured on Mueller-Hinton plates devoid of extract. The lowest level of extract displaying no proliferation of bacteria was considered the MBC [22,23].

2.10. Scanning electron microscopy (SEM)

Scanning electron microscopy was worked for observing the morphological characteristics of the two main foodborne microorganisms *S. aureus* ATCC 25923, and *E. coli* ATCC 25922.

2.11. Fourier transform infrared (FTIR) spectra analysis

Using FTIR to determine the specific chemical groups present in the ultrasonic extract that were produced under ideal processing conditions, which are directly connected to the existence of bioactive constituents, The extract was first ground using a pestle and mortar after being was subjected to dehydration at a temperature of 40 °C under vacuum conditions in an oven. The material was compacted into a disc (2–3 mm) after being combined with potassium bromide (KBr), and its transmission mode in the range of 500–4000 cm^{-1} was recorded [24].

2.12. Statistical analysis

The results gathered through the three experimental replications were used for all statistical analyses. The results were statistically analyzed using analysis of variance (ANOVA) via SPSS statistical software, version 22 (SPSS Inc., Chicago, IL, USA). Duncan's analysis was utilized to determine the variability in mean values. The significant differences ($P < 0.05$) between the means were determined.

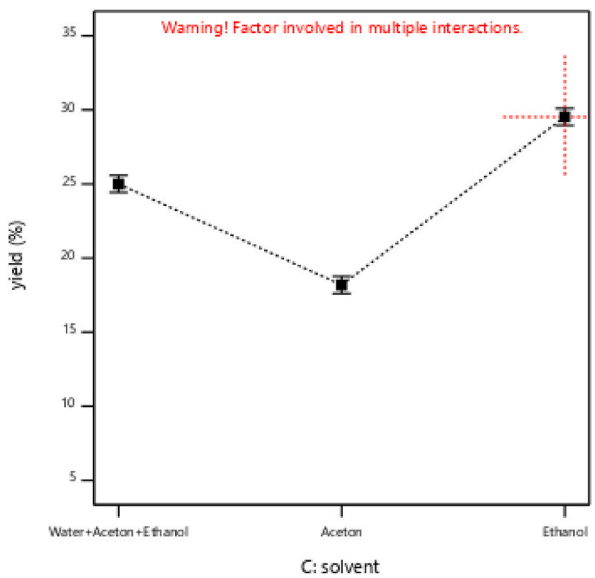

Fig. 1. The effect of solvent type factor on phenolic compounds extraction.

3. Results and discussion

3.1. The effect of solvent type on phenolic compounds extraction

Fig. 1 explains the impact of solvent type on the number of phenolic constituents found in carrot pomace extract. In this investigation, various solvents for extraction were used as follows: water/acetone/ethanol (30:35:35), ethanol 70 %, and acetone 70 %. The highest and lowest extraction percentages were obtained by ethanol 70 % (30 %) and acetone 70 % (about 18 %) solvents, respectively. In this context, Dent and colleagues detected an increase of more than 30 % and as high as 70 % in the volume percentage of acetone or ethanol in water by maceration, leading to a noteworthy decrease in the harvesting efficacy of total polyphenols from common sage [25]. Pang and colleagues investigated the impact of solvent type and separation technique on the polyphenols present in *Orthosiphon stamineus* leaves. Several solvents, including 50 % methanol, 50 % isopropyl alcohol, 70 % isopropyl alcohol, 70 % methanol, isopropyl alcohol, methanol, and water, were used to compare the maceration with 240 min of extraction and the UAE with 90 min of extraction. The results showed that aqueous alcohol was up to 40 % more efficient than a pure solvent. Prolonging the extraction duration to 90 min led to a reduction in the output of polyphenols owing to thermal degradation [7].

3.2. The influence of time on phenolic compounds extraction

The amounts of phenolic constituents extracted from carrot pomace following the ultrasonication time are presented in Fig. 2. As was to be expected, there is a direct relationship between the time factor and the phenolic compound extraction output. The outcomes attained disclosed that the extraction rate of phenolic constituents increased from 0 to 15 min. In accordance with our findings, Ma and colleagues stated that after increasing the sonication time from 20 to 60 min, the concentration of hesperidin obtained from penggan (*Citrus reticulata*) peel in methanolic extracts increased significantly [26]. According to Yingngam and colleagues, the level of phenolic constituents rose as the extraction duration and ultrasonic power increased. The phenolic components of *Cratoxylum formosum* ssp. *formosum* leaves rose meaningfully between 10 and 20 min of extraction, from 38 mg GAE/gr to 41 mg GAE/gr, according to their findings [27].

3.3. The effect of ultrasonication power on phenolic compounds extraction

Fig. 3 displays the result of ultrasonication power on the contents of phenolic constituents. In the present research, three different powers of sonication were used (0, 200, and 250 w). The highest extraction rate of phenolic compounds was observed at 250 powers. In this way, Pan and colleagues assessed the polyphenols of pomegranate peel and stated that 20–100 kHz ultrasonic radiations were efficient for the extraction of bioactive elements and may be used effectively because of their high repeatability, little energy and solvent usage, low extraction temperature utilization, and subsequently less phenolic loss [28]. Due to the degradation of phenolic substances under challenging extraction circumstances, previous studies have suggested that lengthening the period at high ultrasonic powers by up to 20 min can result in a reduction in the antioxidant properties [29].

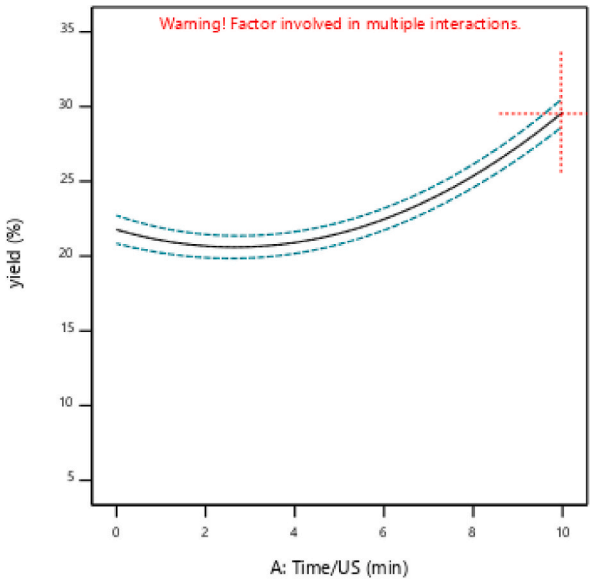

Fig. 2. The effect of time factor on phenolic compounds extraction.

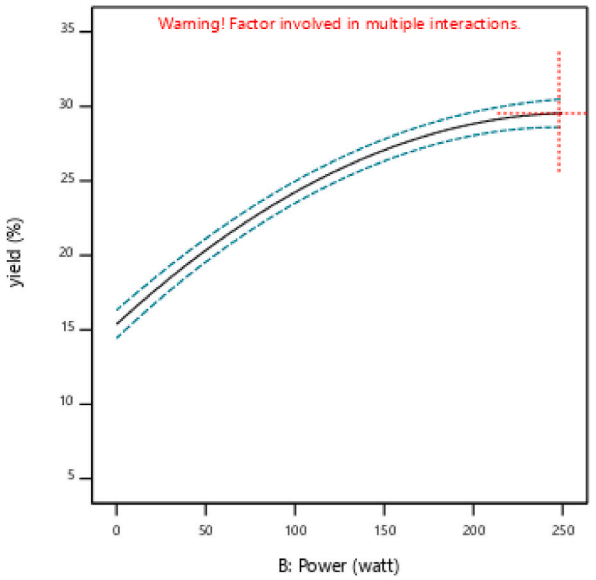

Fig. 3. The effect of ultrasonication power on phenolic compounds extraction.

3.4. Optimization of extraction parameters

Determining the optimal circumstances is significant to achieve the appropriate extraction velocity and content of the intended substance. The results of the variance analysis of phenolic compound extraction in different optimization circumstances are shown in Table 1. The analysis of variance for the elected models demonstrated how well the models explain the connection between the independent and dependent variables in the model. The model's *p*-values were less than 0.0001 and its F-values were more than 291. In comparison to the pure error, the lack of fit test was statistically insignificant. The adjusted and predicted r^2 of the model were high (above 0.98) and in good agreement. This demonstrates that the model is reliable and appropriate for describing experimental results (Table 2). Moreover, the sufficiency of the model was assessed via the residuals, which indicate the difference between the perceived and expected values of the response. The aspects of variance not explained by the regression model are what the residuals are meant to be. The residual vs. run plot presented in Fig. 4A displays that the response is not impacted by any lurking variable, as illustrated by the randomly dispersed points. Likewise, the great lineal pattern detected in the predicted vs. real values of yield (Fig. 4B) approved that the noticed responses were in a decent association with the expected ones.

Since usually the extraction process of these compounds is under the direct influence of various variables and also these elements may have synergistic or antagonistic influences on each other. Therefore, in order to acquire an extract with the highest efficiency and quality, it is necessary to study the optimal values between different factors and determine the best situation. The three-dimensional response surface plots as explained in Fig. 5A and B. Results indicated all three factors, affected polyphenolic compounds extraction. The extraction time plays a key role in polyphenolics because raising the solvent's interaction time (ethanol 70 %) with solid substances, possibly enhance the dispersion of the substances. The ultrasonication can also increase the amounts of these compounds. It is associated with the strength of ultrasonic waves that have the potential to penetrate the matrix and break down cell walls, allowing phenolic chemicals to be released into the reaction medium. Overall, the cavitation phenomenon is the leading mechanism

Table 1
Analysis of variance (ANOVA) of the quadratic response surface model.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1267.06	11	115.19	291.14	<0.0001	Significant
A-Time/US	566.72	1	566.72	1432.41	<0.0001	
B-Power	37.56	1	37.56	94.92	<0.0001	
C-solvent	30.82	2	15.41	38.95	<0.0001	
AB	0.0833	1	0.0833	0.2106	0.6499	
AC	23.44	2	11.72	29.63	<0.0001	
BC	434.78	2	217.39	549.46	<0.0001	
A^2	144.64	1	144.64	365.59	<0.0001	
B^2	91.43	1	91.43	231.09	<0.0001	
Residual	10.68	27	0.3956			
Lack of Fit	7.48	15	0.4988	1.87	0.1399	Not significant
Pure Error	3.20	12	0.2667			
Cor Total	1277.74	38				

Table 2
Model fit statistics.

Std. Dev.	0.6290	R^2	0.9916
Mean	16.82	**Adjusted R^2**	0.9882
C.V.%	3.74	**Predicted R^2**	0.9814
		Adeq Precision	68.8229

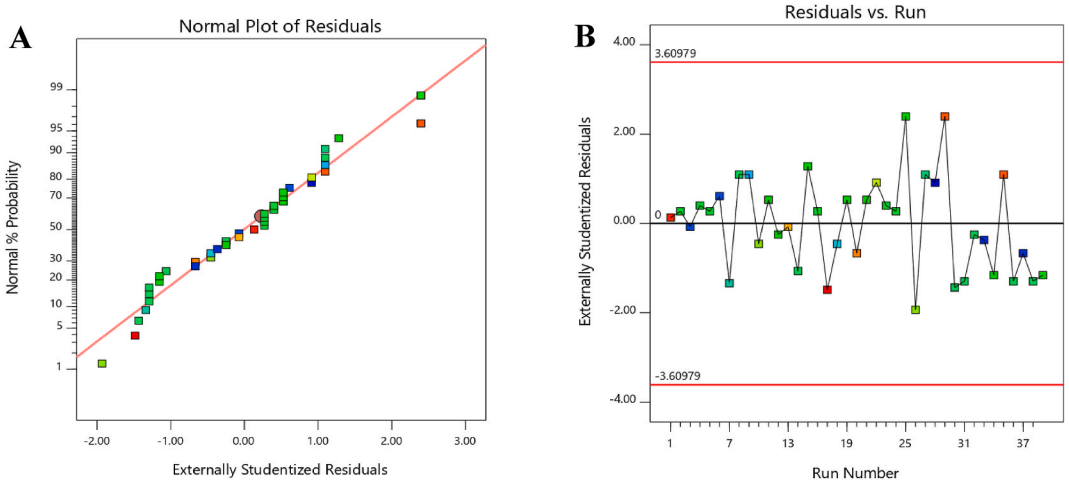

Fig. 4. (A) Externally studentized residuals vs. run number plot; and (B) Predicted vs. actual values plot for yield.

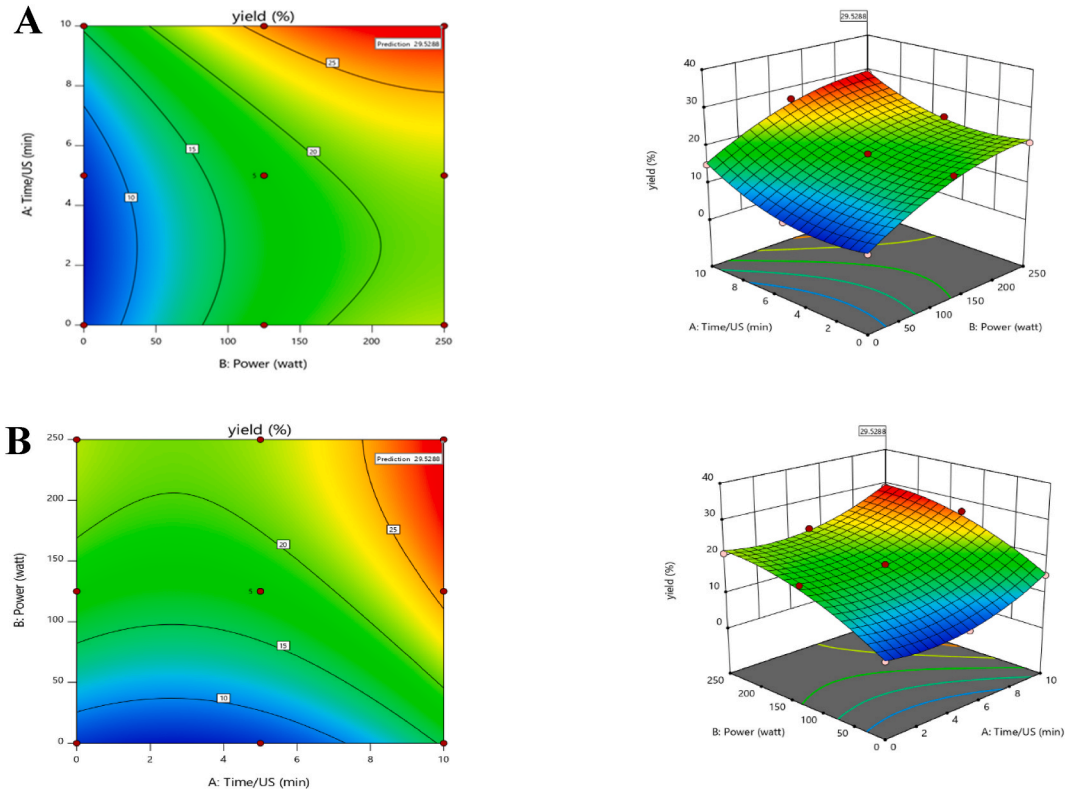

Fig. 5. Response surface plots of interaction effects of (A) time and (B) ultrasonication power factors on yield extraction.

accountable for the intensification of the ultrasound-based extraction process. The shockwave-persuaded disintegration of the plant cell wall promotes the mass transmission of phenolics via cell membranes in the solution after the cavitation blobs shatter at the interface of the plant cells [2,30]. However, some studies have shown that ultrasonic waves lead to the degradation of several phenolics and the generation of extremely hypersensitive radicals into the gas blobs [31,32]. In the study performed by Zhu and colleagues, they gained 13 substances with UAE and only 6 substances through regular solvent extraction. The ultrasound waves exhibited the successful capacity to lead to greater recovery of valuable elements via cavitation by increasing cell disruption, solvent permeation, and mass transmission [33]. Ghasemzadeh and colleagues concluded the most suitable set of situations for the extraction of phenolics from curry leaves was an ultrasonic power of 145.49 w at 55.9 °C for 20 min with an 80 % methanol solvent [32]. Besides, Moeini and colleagues defined the optimal circumstances for extracting phenolic constituents from *Agrimonia eupatoria* as follows: ultrasonic power: 100 w, extraction time: 41.82 min, and the ratio of ethanol to water: 1.17 v/v [33]. Skiba and colleagues (2018) investigated the kinetics and thermodynamics of the plasmochemical generation of silver nanoparticles. The study concluded that the mean dimension of particles created using plasmochemical methods is influenced by the initial level of silver ions in the solution. The mean diameter ranges from 36.5 to 60.1 nm for concentrations of silver ions ranging from 0.25 to 3.0 mmol per liter. Additionally, it has been discovered that the plasmochemical production of silver nanoparticles follows a second-order process. The constant rate for the creation of silver particles is k = 0.07–1.53 mol-1•dm3•min-1, which varies based on the initial level of silver ions. It is demonstrated that the peak λ_{max} = 400–440 nm is present when silver nanodispersions are formed under the influence of a plasma discharge [34]

3.5. Extraction yield (%)

Fig. 6A and B represents the extraction yield (%) of carrot pomace obtained by three distinct solvents (water/acetone/ethanol, acetone, and ethanol) at three different times (0, 5, and 10 min), with three various powers (0, 200, and 250 w). The highest yield (about 30 %) was determined at 70 % ethanol at 10 min. Additionally, it was noted that carrot pomace extracted more yield at 70 % ethanol with a power of 250 w.

3.6. Total phenolic content (TPC)

The TPC of the optimal sample was obtained at 85 mg of gallic acid per gram of extract. A plant extract's antioxidant capacity is influenced by a number of variables, such as the nature and charge of the solvent, the purity of active constituents, the isolation procedures, and the assessment system applied to determine the activity [14]. In this regard, Wang and colleagues described that for the extraction of polyphenolic elements from blueberry leaves utilizing ultrasound negative pressure cavitation, the most suitable ethanol range was 60–70 %. These researchers found that TPC and TFC (total flavonoid content) augmented as ethanol levels rose from 40 % to 70 %, but extraction efficiency dropped as ethanol concentrations increased further, up to 90 % [35]. Additionally, Khoshdouni Farahani and colleagues investigated the extraction efficacy and content of phenolic compounds in jujube fruit (*Ziziphus* spp.) by ultrasonication and maceration procedures. The findings disclosed that the ultrasonic bath extraction procedure, 80 % ethanol solvent, 60 min, and 50 °C extracted the utmost content of phenolic constituents from the fruit, and the effectiveness of harvesting of its extract was 95.66 %. In the case of maceration extraction, 80 % ethanol solvent extracted the utmost content of phenolic compounds, and the extraction efficiency of the extract was reported at 77 % [36]. The findings of our study were consistent with the results of prior research, which came to the conclusion that UAE may be regarded as an effective method to extract bioactive components from polyphenols [37]. In a carrot, the phenolic element is greater in the peel than in the core. Only 39.5 % and 6.4 % of the total phenols are found in the xylem and phloem tissues, respectively; 54.1 % are found in the peel. Consequently, compared to whole carrots, a higher phenolic content has been reported in carrot puree [38]. According to the preliminary results obtained from the pilot evaluations

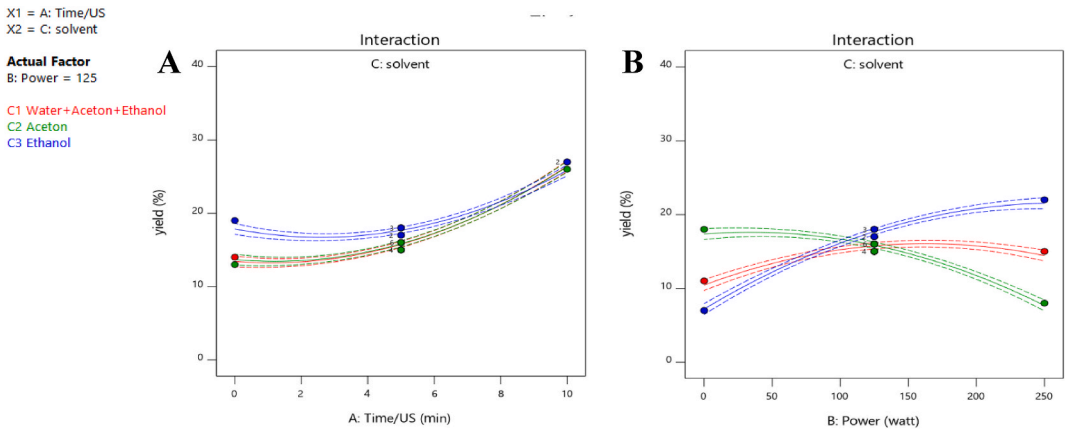

Fig. 6. Diagram of the interaction effects of solvent type, (A) extraction time, and (B) power on the extraction yield (%).

(unpublished data), the determined total carotenoid of the carrot pomace was 12.20 μg/g. On the other hand, the investigated carrot pomace showed a high phenolic content (85 mg of gallic acid per gram of extract). Therefore, it can be acknowledged that it mediates a major part of carrot pomace biological activities. Likewise, it has been reported that black carrot peels and black carrot pomace had higher polyphenol percentages compared to whole black carrots, probably because cellular constituents like cellulose and cellulose-pectin aggregates break down and liberate bound substances [39]. Similarly, the comparison of soluble and insoluble-bound phenolics of various byproducts generated by industrial facilities showed that the bound phenolics of pomegranate peel, pomegranate seed, apple pomace, chestnut shell, black carrot pomace, and apple peel were 16 %, 25 %, 45 %, 47 %, 53 %, and 88 % of TPC, respectively [40].

3.7. DPPH radical scavenging

It is known that DPPH is a chemical compound having a proton-free radical that declines meaningfully when exposed to scavengers of proton radicals [41]. The antiradical efficacy of carrot pomace extract was evaluated using the DPPH test. The optimized sample showed a high percentage of inhibition (EC_{50}: 55 ± 1 μg/mL).

3.8. Reducing power

The reduction of Fe (III) (Fe^{3+}) is frequently used as a function indication for electron donation, which is an imperative mode of the phenolic antioxidant attribute [19]. The outcomes on reducing power demonstrate the improved carrot pomace extract's ability to operate as an electron donor, reducing free radicals by producing stable intermediates. Reducing the power of the tested sample by 22 %. Gulsunoglu and colleagues investigated the antioxidant activity of different industrial factory wastes. Compared to other wastes, pomegranate peel and chestnut shell presented higher antioxidant properties. Although the maximum antioxidant activity was related to the soluble phenolic extract of pomegranate peel, the bound phenolic extract of the chestnut shell showed the maximum antioxidant function among relevant extracts of the wastes [40]. Nevertheless, antioxidant compounds display their antioxidant activity using different mechanisms, among which are the avoidance of chain initiation, coupling of transition metal ion catalysts, degradation of peroxide radicals, inhibition of continued hydrogen abstraction, reductive capability, and radical scavenging [42]. Several investigations have shown a clear correlation between antioxidant properties and reducing power in specific plant extracts, which is linked to the antioxidant potential of such extracts [43–46]. Vasyliev and colleagues (2020) investigated the capacity of fruit pomace preparations to reduce substances and their potential to act as antioxidants using spectrophotometric and electrochemical techniques and found that the grape extract exhibited a greater reducing capacity than the apricot and black currant preparations in the potassium ferricyanide reduction (FRAP) and phosphomolybdenum techniques [47]. In this way, the investigated carrot pomace, in addition to antioxidant activity, possesses a significant electron-donating activity (EC_{50}: 55 ± 1 μg/mL), so it can be stated that in the case of carrot pomace, antioxidant and lowering power actions are closely related.

3.9. Antimicrobial activity

The results of the antimicrobial function of carrot pomace extract are illustrated in Table 3. All specimens were discovered to be effective toward both *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, with inhibition zones between 8.5 and 12 mm for *S. aureus* and between 7.5 and 11 mm for *E. coli* at the considered levels (550, 650, and 750 mg/mL). By increasing the concentration of the extract, the inhibitory effect also increased, and the highest antimicrobial activity was noticed at a concentration of 750 μg/mL.

3.9.1. Minimum inhibitory concentration (MIC)

The MIC results of carrot pomace extract are given in Table 4. Our findings indicated that carrot pomace extract had been effective at just a concentration of 600 mg/mL on two tested bacteria. Generally, Gram-negative bacteria, like *E. coli*, possess minimal sensitivity to polyphenols in comparison to Gram-positive bacteria, such as *S. aureus*, *Bacillus cereus*, etc., due to the fact that these substances repel the surface lipopolysaccharide of Gram-negative bacteria [48].

3.9.2. Minimum bactericidal concentration (MBC)

As shown in Table 5, the carrot pomace extract was effective at a level of 600 mg/mL and higher on both tested bacteria. Vodnar and colleagues reported that among Gram-positive bacteria, in comparison with *S. aureus*, *Bacillus cereus* exhibited a higher resistance [49].

Regarding the potential antimicrobial effect, in comparison to *E. coli*, carrot pomace extract showed a higher antimicrobial effect on

Table 3
Antimicrobial activity of carrot pomace extract against *Staphylococcus aureus* and *Escherichia coli*.

Extract concentration (mg/mL)	Inhibition zone (mm)	
	Staphylococcus aureus	Escherichia coli
550	8.5	7.5
650	10	11
750	12	11

Table 4
Minimum inhibitory concentration (MIC) of carrot pomace extract against *Staphylococcus aureus* and *Escherichia coli*.

Extract concentration (mg/mL)	Inhibition zone (mm)	
	Staphylococcus aureus	Escherichia coli
Control	–	–
200	–	–
300	–	–
400	–	–
500	–	–
600	+	+

Table 5
Minimum bacterial concentration (MBC) of carrot pomace extract against *Staphylococcus aureus* and *Escherichia coli*.

Extract concentration (mg/mL)	Inhibition zone (mm)	
	Staphylococcus aureus	Escherichia coli
Control	–	–
200	–	–
300	–	–
400	–	–
500	–	–
600	+	+

S. aureus. According to the antimicrobial assay, by boosting the extract's concentration, the inhibitory effect also increased, and the highest antimicrobial function was detected at the concentration of 750 μg/mL. Following our findings, Cheaib and colleagues described that carrot pomace exhibited more anti-bacterial action than orange pomace. In contrast to orange pomace, which only had antibacterial action against two Methicillin-resistant *S. aureus* Gram-positive strains, carrot pomace extracts inhibited two Methicillin-resistant *S. aureus* Gram-positive strains as well as Enterococci (HLARVRE) strains [50]. In order to achieve an inhibitory zone using the disc-diffusion approach for *Pseudomonas aeruginosa*, *S. aureus*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *E. coli*, Nirmala and Narendhirakannan additionally employed greater quantities of grape skin extract within a spectrum of 50–250 mg/mL. Based on the MIC findings, carrot pomace extract had been effective at just a concentration of 600 mg/mL on two investigated bacteria [51]. In Cheng and colleagues' study, the relationship between antimicrobial activities and TPC versus *Candida albicans* and *S. aureus* was feeble but positively significant (r = 032 and 0.58, respectively), showing that phenolic constituents could be contributing to the antibacterial properties versus *Candida albicans* and *S. aureus,* while a significantly feeble and negative relationship between TPC and antimicrobial function versus *E. coli* (r = 0.38) was observed [52]. In the same way, Alizadeh Behbahani and colleagues reported that the MIC values of the aqueous preparation of *Eucalyptus camaldulensis* leaves for *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* were 8, 64, and 32 mg/mL, respectively. *Streptococcus pyogenes* responded favorably to the aqueous preparation of *Eucalyptus camaldulensis* leaves, whereas *Pseudomonas aeruginosa* was the least affected. Vorobyova and colleagues (2022) researched to investigate the use of silver nanoparticles (Ag-NPs) in enhancing the photocatalytic capacity of commercially available TiO_2 (P25) and the antibacterial properties of surgical sutures. This study presents a novel technique for the environmentally friendly production of silver nanoparticles using a water-based grape skin extract. The grape skin preparation is first treated with oxygen and ultrasonic to prepare it for the process of synthesis. The sutures exhibited bacteriostatic and antifungal properties against Candida albicans, Gram-negative (*E. coli*), and Gram-positive (*Bacillus subtilis*) wound infections. The study found that the process of the electrochemical implantation of Ag-NPs on nylon surgical staples did not change the mechanical characteristics of the sutures, but it did provide them with antibacterial capabilities. When compared to TiO_2 change using the impregnation method, the antibacterial activity experiments' findings demonstrated that TiO_2 modified utilizing the green approach displayed greater antibacterial action versus Gram-negative bacteria [53]. Regarding the MBC assay, the carrot pomace extract was effective at a level of 600 mg/mL and higher on both investigated bacteria. The utmost sensibility of *B. cereus* was to RGT (as red-grape waste thermally processed) (MIC: 3.9 and MBC: 7.81 mg/mL), RGF, and WGT (white-grape waste thermally processed) (MIC: 7.81 and MBC: 15.62). In regard to *E. faecalis*, the MIC recorded was 7.81 mg/mL for AT (apple waste thermally processed) and WGT extracts. The following extracts, RGF, BF, RGT (redbeet waste fresh) with a MIC and MBC of 1.953 and 3.9 mg/mL, respectively, and WGT, showed significant inhibitory activity against *E. coli* [54].

3.9.3. Scanning electron microscopy

Micrographs of the tested bacteria in the study, *S. aureus* and *E. coli,* are presented in Fig. 7A and B. *S. aureus* cells become visible in a spherical shape. When seen under a light microscope following Gram staining, they are typically in clusters that resemble a bunch of grapes. Greek words that translate to "bunch of grapes (staphyle) and berries (kokkos)" are the source of the term "Staphylococcus." Cells with smooth surfaces and an approximately spherical form are visible by scanning electron microscopy [55]. *E. coli* is a normal rod-shaped, gram-negative bacterium with rounded ends. Nevertheless, the actual form of these bacteria differs from spherical (Cocci) cells through elongated or filamentous rods. This is arranged singly or in pairs. *Escherichia coli* is non-spore-forming and is typically

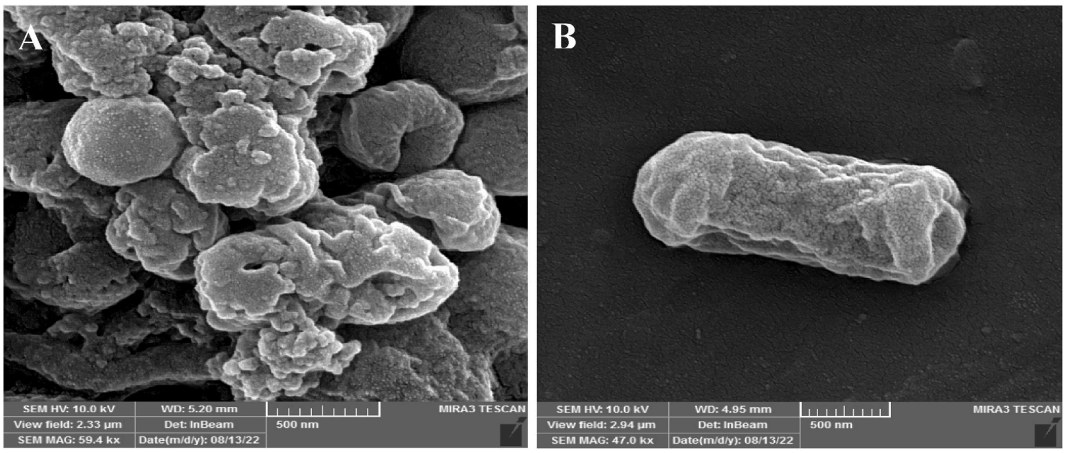

Fig. 7. Micrographs of tested bacteria in the study, *Staphylococcus aureus* (A) and *Escherichia coli* (B).

motile due to peritrichous flagella [56].

3.10. Fourier transform infrared (FTIR) spectra

FTIR is a well-known molecular absorption spectrum that determines the capacity to alter energy levels on the basis of molecular vibrations. FTIR analysis was carried out to determine the function of biomolecules in the optimal example (500–4000 cm^{-1}) (Fig. 8). Polygalactronic acid compounds include pectins and similar compounds. The bands seen at 3359 cm^{-1} unambiguously relate to hydroxyl (–OH) groups, whereas the signal at 2926 cm^{-1} corresponds to carbon-hydrogen (C–H) bonds. Pectin in the specimen is the reason for the stretching (saturation) of $–CH_2$ groups. The presence of the band at 1634 cm is attributed to the symmetric and asymmetric fluctuations associated with ionized carboxyl groups. Conversely, the band at 1666 cm^{-1}, which originates from esterified carboxylate groups, is almost nonexistent. The bands seen at 1414, 1453, and 1260 cm-1 are attributed to the presence of –C–O–C groups in the polygalactanic acid chain. Additionally, the bands at 1138, 1102, 1052, and 990 cm^{-1} also belong to the polygalactanic acid chain, which aligns with the findings of Lee and colleagues [57].

4. Conclusion

In summary, vegetable and fruit pomaces are the residual materials generated by agro-fruit sectors after the production of juice. Nevertheless, they contain various bioactive constituents, such as vitamins, proteins, phenolic elements, carbohydrates, dietary fiber, pectin, and minerals. Thus, the current investigation was carried out with the aim of extracting valuable polyphenolic compounds from carrot pomace and investigating its potential antioxidant and antimicrobial properties. Ultrasound-assisted extraction is a non-thermal, efficient, and economic extraction procedure. Nevertheless, the elements connected with UAE, including power, the cycle of work, time, temperature, frequency, type of solvent, and ratio of liquid to solid, must be assumed and optimized for each food or by-product. The following were the UAE's optimal settings for the current study: 10 min were allotted for extraction, 250 w of ultrasonic

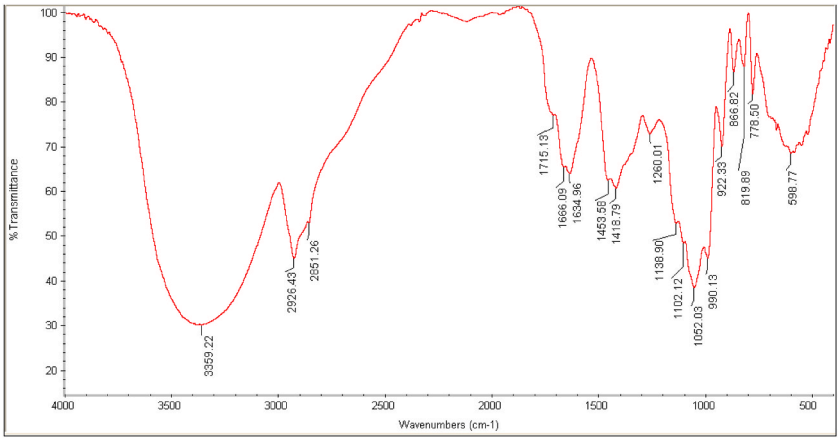

Fig. 8. FTIR spectra of the optimal sample.

power was used, and the solvent used was 70 % ethanol. The maximal levels of TPC and DPPH radical scavenging under these circumstances were 85 mg GAE/gr and EC_{50}: 55 ± 1 μg/mL, respectively. The finding revealed that carrot pomace is a plentiful source of antioxidant substances that can be utilized to boost food items' nutritional value. However, the precise chemical profile of polyphenolic compounds can be investigated in future studies. As a final point, due to the high antioxidant and antimicrobial activity of many agro-industrial waste extracts, they are beneficial to use as sources of food additives intended to prolong the shelf life and ensure the safety of food goods.

Data availability statement

All data used have been included.

CRediT authorship contribution statement

Sahar Sabahi: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Amin Abbasi:** Writing – review & editing, Formal analysis. **Seyed Ali Mortazavi:** Writing – review & editing, Resources, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

CRD	Composite rotatable design
IFRF	Insoluble fiber-rich fraction
PEF	Pulsed electric field
UAE	Ultrasound-assisted extraction
MAE	Microwave-assisted extraction
HVED	High voltage electrical discharges extraction
SFE	Supercritical fluid extraction
PFE	Pressurized fluid extraction
EAE	Enzyme-assisted extraction
HPAE	High pressure-assisted extraction
DPPH	2,2-Diphenyl-1-picrylhydrazyl
TPC	Total phenolic content
DD	Disk diffusion
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
SEM	Scanning electron microscopy
FTIR	Fourier transform infrared
TFC	Total flavonoid content
RGT	Red-grape waste thermally processed
WGT	White-grape waste thermally processed
AT	Apple waste thermally processed

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