Characterization of *Feijoa sellowiana* leaves based on volatile and phenolic compound compositions and antimicrobial properties

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Abstract

In the present research study, response surface methodology (RSM) was performed on the basis of total phenolic content of aqueous feijoa leaf extracts. The total phenolic content of experimental runs changed between 941.6 and 4347.5 mg GAE/L. HPLC-DAD analysis was performed to monitor the phenolic profile of feijoa leaf extract. Major phenolic compounds were determined as gallic acid, catechin, syringic acid, ellagic acid, chrysin, caffeic acid, *p*-coumaric acid, ferulic acid, and quercetin. Profile of volatile compounds was determined by using GC-MS technique and limonene, linalool and caryophyllene were detected as major volatile compounds. In addition, extracts from feijoa leaves showed antimicrobial activity against Gram-positive and Gram-negative bacteria and one yeast species. Taken together, all findings enhance our understanding of further use feijoa leaves as potential source for valuable bioactive compounds in various industrial products such as dietary supplements, food products (syrups, jams), and cosmetic products.

Keywords: feijoa leaves; RSM (response surface methodology); volatile profile; phenolics; antimicrobial.

Practical Application: Implementation of RSM for maximization of the extraction of total phenolics from the feijoa leaf using the factors of extraction temperature, time, concentration, and the shaking type.

1 Introduction

Feijoa sellowiana, which is a species of the family Myrtaceae is mainly grown in South America, Brazil, and New Zealand (Weston, 2010). The feijoa is known as guavasteen pineapple. Guava produces its fruits in early fall and late summer (Bimakr et al., 2019). Turkey is the homeland of a wide variety of fruits due to its favorable geographic and climate conditions. Feijoa is mainly cultivated in Sakarya, Aydın, and İzmir in Turkey (Beyhan et al., 2016). Feijoa fruit involves a wide range of healthy compounds such as dietary fibre, vitamins, and antioxidants. Also, by-products of feijoa were reported to have functional ingredients such as phytochemicals and fibres. Previous investigations reported that feijoa extracts showed beneficial effects such as anti-microbial activity, anti-cancer activity, anti-inflammatory activity, immunity-stimulating activity, and anti-oxidant activity (Weston, 2010). In addition, most of the previous reports focused on the antioxidant properties of feijoa fruit (Zhen-Zhu et al., 2011; Mosbah et al., 2018). Data from several studies suggest that fruits and leaves of feijoa have valuable phytochemicals such as flavonoids, tannins, saponins, and terpenes in their compositions (Bimakr et al., 2019). Additionally, feijoa leaves were reported to have bioactive compounds in their compositions. However, leaves were regarded as waste and further use wasn't defined for them. Very few studies focused on exploring feijoa leaves in terms of their bioactive, antimicrobial, and antioxidant properties. The study of Poodi et al. (2018) attempted to optimize the ultrasound-assisted extraction of bioactive compounds from feijoa

leaves in Iran (Poodi et al., 2018). Beyhan et al. (2010) assessed the total phenolic compounds and antioxidant capacity of feijoa leaf, dry fruit, and fresh fruit (Beyhan et al., 2010). In another study, antioxidant characteristics of feijoa leaves extract were determined by spectrophotometric methods (Ming et al., 2012). Mosbah et al. (2018) comprehensively evaluated the antioxidant, antimicrobial and pharmacological properties of feijoa leaves from Tunisia. In the previous studies, it was shown that feijoa fruit extract showed high antimicrobial and antifungal properties (Mosbah et al., 2018). It was presented that RSM has been used effectively for optimization of extraction parameters of phenolics from a wide variety of foods and agricultural products. Şahin & Şamlı, (2013) determined extraction conditions for ultrasound-assisted extraction of phenolics from olive leaf extract using RSM (Şahin & Şamlı, 2013). Another previous study used RSM for optimization of extraction of phenolics from *Inga edulis* leaves (Silva et al., 2007).

As can be seen, up to now, very little research has been carried out on feijoa leave around the world. In the current research, we applied RSM (response surface methodology), for the first time, for optimization of aqueous extraction of phenolics from feijoa leaves. For this purpose, the effects of four factors solid(feijoa)/solvent(water) ratio, extraction temperature, extraction time, and shaking type were evaluated to find extraction condition which would maximize total phenolic content (TPC) of aqueous

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extracts of feijoa leaves. To the best of our knowledge, this is the first attempt for the evaluation of feijoa leaves from Turkey. In our research study, we optimized the extraction of phenolics from feijoa leaves using response surface methodology. Volatile content and phenolics profile were determined by using GC-MS and HPLC techniques, respectively. Antimicrobial activity was determined against selected pathogenic microorganisms.

2 Materials and methods

2.1 Apparatus and reagents

The feijoa leaves were dried by using an oven (Memmert IN110) at 40 ± 3 °C for 24 h. Dried leaves were ground by using a grinder machine (Fakir, Germany) for 30 s to obtain a homogenous powder which can pass through a stainless steel sieve (mesh 595 μ m). Aqueous extracts of feijoa leaves were obtained using vertical and horizontal shakers. Total phenolics concentrations were determined by using a UV-Vis spectrophotometer (Shimadzu UV-1800, Japan). HPLC profile was determined by using Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) composed of pump (LC-20AD), oven (CTO-10ASVP), autosampler (SIL-20A HT), and diode array detector (SPDM20A). Volatile content of feijoa extract was determined by using the GCMS-QP2010 gas chromatography-mass spectrometry system (Shimadzu, Milan, Italy). Folin Ciocalteu's phenol reagent, gallic acid standard, acetic acid, and acetonitrile were obtained from Sigma-Aldrich and Merck. Feijoa leaves were collected in November 2019 in Sakarya (Turkey).

2.2 Extraction of phenolic compounds

Dried feijoa leaves were ground using a grinder (Fakir Aromatic, Germany). Extractions were performed according to the Design Expert (Design-Expert Software (Stat-Ease Inc., Version 11. Minneapolis, USA). The design model included 40 experimental steps as it is shown in Table 1. Factors of the model were temperature (°C), time (min), feijoa concentration (g/mL), and shaking style (horizontal or vertical). Extraction temperature (25–100 °C), time (5–30 min) and feijoa concentration (25–100 mg/mL) were shown for each batch. In the extraction process, only aqueous solutions of feijoa leaves were prepared according to the experimental pattern in Table 1. Extracts were centrifuged at 5000 xg for 15 min and samples were filtered through an ordinary filter paper (Whatman, thickness: 1mm). The filtrate was collected in a glass tube and stored at 4 °C in a refrigerator until further use.

2.3 Determination of Total Phenolic Content (TPC)

Total phenolics content of the feijoa extracts, which were extracted according to the experimental run (Table 1), were determined using Folin-Ciocalteu (FC) method following the previous studies (Cebi et al., 2019). 2.5 mL of FC reagent and 2 mL of sodium carbonate solution (7.5%) were added to 0.5 mL of diluted feijoa extract. Samples were incubated in the dark at room temperature for 30 min. Afterwards, absorbance was measured at 760 nm using a UV-Visible spectrophotometer. Obtained results were presented as mg gallic acid equivalents per liter (mg GAE/L) of feijoa extract.

2.4 HPLC analysis of feijoa extract

HPLC analysis of feijoa extract was attained with some modifications in the previous studies (Kim et al., 2008). A HPLC coupled diode array detector (HPLC-DAD) system was used in experiments. HPLC system (Shimadzu) included pump, detector, autosampler, oven, degasser, and communications bus module (Shimadzu Corp., Kyoto, Japan). Standard calibration curves were prepared by using the analytical grade standards of gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, caffeic acid, chlorogenic acid, syringic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, rutin, ferulic acid, myricetin, quercetin, kaempferol, and chrysin (Sigma-Aldrich). Quite linear R^2 values of 0.999 were obtained for all included standards between the concentration range of 1.95 and 156.25 ppm (mg/L). A C-18 reversed-phase column (GL Sciences, Intersil® ODS, 250 mm $\times$ 4.6 mm $\times$ 5 μ m) was used for separation phenolic compounds at 40 °C oven temperature. The selected wavelengths were 280, 290, 320, and 360 nm for UV detection. Mobile phase A (distilled water with 0.1% acetic acid) and mobile phase B (acetonitrile with 0.1% acetic acid) were used for separation at 1 mL/min flow rate. The injection volume was 20 μ L. The gradient was 10% B (0–2 min), 10–30%B (2–27 min), 30–90% B (27–50 min), and 90–100% B (51–60 min). HPLC-DAD results are presented as mg/g feijoa leaf.

2.5 Static HS-GC/MS analysis of feijoa leaves

Feijoa leaves were ground by a spice grinder machine (Fakir Aromatic, Germany). 4 g of ground feijoa leaves were placed in 20 mL headspace vials. Samples were heated and agitated at 70 °C for 15 min by the autosampler system. 500 rpm agitation speed was implemented with 500 μ L injection volume.

Head space/gas chromatography-mass spectrometry analysis of feijoa leaves was performed with slight modifications in the previously described methodology of Cebi et al. (2019). GCMS-QP2010 (Shimadzu, Milan, Italy) combined with a CTC-Combi-PAL-autosampler was used in the head space/gas chromatography-mass spectrometry analysis. Separation of volatile compounds were obtained by using Rtx-5MS fused silica capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μ m) with the carrier gas Helium. The oven temperature was set as the following procedure: 40 °C (3 min); 40–176 °C (8 °C/min); and 176 °C (20 min). The injector temperature, pressure value, and the linear velocity were 150 °C, 95.8 kPa and 47.1 cm/s, respectively.

Interface temperature and ion source temperature were programmed as 280 °C and 230 °C, respectively. A mass range of 35–550 *m/z* and a detector voltage of 70 eV were employed for the mass spectroscopy analysis of samples. Spectral identification of volatile compounds was performed by using the libraries of the GC-MS instrument. The volatile composition of the leaf was determined by computer matching of scanned mass spectra of feijoa samples with commercial mass spectra libraries (NIST27 and WILEY7). The quantitative amount of each volatile compound was determined on the basis of the calculation of relative peak area to the total peak area.

2.6 Antimicrobial activity analysis of feijoa leaves

Antimicrobial activity of feijoa leaves was determined using the agar diffusion method as described previously (Sagdic et al.,

Table 1. Experimental design, factors, actual and predicted total phenolics of response surface model (RSM).

Experiment (Run)	Factor 1: Temperature (°C)	Factor 2: Time (min)	Factor 3: Concentration solid (feijoa) / solvent (water) ratio (mg/mL)	Factor 4: Shaking Type (vertical, horizontal)	Actual Total Phenolics (mgGAE/L)	Predicted Total Phenolics by RSM model (mgGAE/L)
1	62.5	5	62.5	vertical	2480.49	2291.06
2	25	30	100	horizontal	3151.22	3064.23
3	62.5	17.5	62.5	horizontal	2274.80	2432.47
4	100	30	100	vertical	4026.02	3955.93
5	62.5	17.5	62.5	vertical	2169.51	2236.57
6	62.5	17.5	62.5	vertical	2193.09	2236.57
7	25	5	100	vertical	3057.32	3073.28
8	100	5	100	vertical	4347.56	4455.44
9	62.5	17.5	62.5	vertical	2229.27	2236.57
10	62.5	17.5	62.5	vertical	2220.82	2236.57
11	25	30	25	vertical	1023.17	950.23
12	100	5	25	horizontal	1492.68	1615.63
13	100	5	25	vertical	1640.24	1600.80
14	62.5	17.5	62.5	horizontal	2272.36	2432.47
15	25	5	25	horizontal	970.73	988.84
16	100	30	100	horizontal	3388.21	3406.06
17	62.5	17.5	25	horizontal	1704.07	1507.82
18	25	30	100	vertical	2403.66	2478.04
19	62.5	17.5	62.5	vertical	2249.59	2236.57
20	25	5	25	vertical	1056.10	1161.13
21	25	17.5	62.5	vertical	2372.76	2250.32
22	62.5	17.5	62.5	vertical	2077.64	2236.57
23	62.5	17.5	100	vertical	3248.78	3120.65
24	62.5	5	62.5	horizontal	2260.98	2103.21
25	62.5	30	62.5	horizontal	2044.31	2058.25
26	100	17.5	62.5	vertical	3226.42	3209.10
27	100	30	25	horizontal	1296.75	1274.12
28	25	5	100	horizontal	2754.07	2812.65
29	62.5	30	62.5	vertical	1886.18	1935.85
30	62.5	17.5	62.5	horizontal	2328.00	2432.47
31	62.5	17.5	62.5	horizontal	2406.00	2432.47
32	62.5	17.5	100	horizontal	3433.26	3485.69
33	62.5	17.5	62.5	horizontal	2451.00	2432.47
34	62.5	17.5	62.5	horizontal	2575.00	2432.47
35	100	30	25	vertical	1466.67	1485.64
36	25	30	25	horizontal	1309.76	1387.58
37	62.5	17.5	25	vertical	941.06	929.43
38	100	17.5	62.5	horizontal	2911.38	2835.08
39	25	17.5	62.5	horizontal	2418.29	2350.77
40	100	5	100	horizontal	3936.59	3894.72

2006). Strains were obtained from type culture collections of the Food Engineering Department, Chemical and Metallurgical Faculty at Yildiz Technical University, Turkey. Ten different pathogenic bacteria and one pathogenic yeast were chosen as test organisms were as follows; *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Streptococcus mitis*, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Listeria monocytogenes* ATCC 13932, *Klebsiella pneumoniae* ATCC 43816, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* O157:H7 ATCC 43888, and *Candida*

albicans ATCC 10251, respectively. Before the determination of antimicrobial activity, feijoa leaves were extracted using ethanol-aqueous solvent (80% v/v). The extract was prepared with some modifications on the previous reports (Katalinic et al., 2013). The mixture of ten grams feijoa leaves and 100 mL of ethanol-aqueous solvent (80% v/v) was incubated by shaking for 2 h at room temperature. After incubation, the extract was filtered with Whatman filter paper No. 1 and filtrate was dried under a vacuum using a rotary evaporator at 60°C and lyophilized to complete dryness. All pathogenic microorganisms, which were

Table 2. RSM model statistics and ANOVA analysis.

Source	$R^2_{adj.}$ ^b	$R^2_{pre.}$ ^c	p-value	Lack of fit, ^d p-value	
Reduced Cubic Model	0.9759	0.9090	<0.0001	0.0580	
Linear	0.8880	0.8598	<0.0001	0.0001	
2FI	0.9201	0.8711	0.0126	0.0005	
Quadratic	0.9480	0.8823	0.0026	0.0031	
Cubic	0.9753	0.7541	0.0079	0.0375	
Source	Sum of Squares	DF ^e	Mean Square	F-value	p-value ^f
Model ^a (reduced cubic model)	26960000	19	1419000	84.1	< 0.0001
A-extraction temperature	2603000	1	2603000	154.26	< 0.0001
B-extraction time	200200	1	200200	11.86	0.0026
C-feijoa concentration	21730000	1	21730000	1287.52	< 0.0001
D-shaking type	162400	1	162400	9.62	0.0056
AB	103800	1	103800	6.15	0.0221
AC	488400	1	488400	28.94	< 0.0001
AD	281400	1	281400	16.68	0.0006
BD	120300	1	120300	7.13	0.0147
A ²	587400	1	587400	34.81	< 0.0001
B ²	310000	1	310000	18.37	0.0004
ABD	174700	1	174700	10.35	0.0043
A ² D	152200	1	152200	9.02	0.007
C ² D	104600	1	104600	6.2	0.0217
Residual	337500	20	16874.77		
Lack of fit	249400	10	24942.05	2.83	0.058
Pure Error	88075.01	10	8807.5		
Cor Total	27300000				

^aThe Model *F*-value of 84.10 implies the model is significant. There is only a 0.01% chance that an *F*-value this large could occur due to noise; ^bThe Predicted R^2 of 0.9090 is in reasonable agreement with the Adjusted R^2 of 0.9759; i.e. the difference is less than 0.2; ^cThe lack of fit *p*-value of 0.058 indicate that lack of fit is not significant; ^dDegrees of freedom; ^e*p*-values less than 0.05 indicate model terms are significant. In this case A, B, C, D, AB, AC, AD, BD, A², B², ABD, A²D, C²D are significant model terms.

stored at -80 °C, were inoculated in 1% ratio in Tryptic Soy Broth (Merck-Darmstadt, Germany) and incubated at 37 °C for 24 h. Tryptic Soy Agar (Merck-Darmstadt, Germany) was prepared and autoclaved at 121 °C for 15 min. After autoclave, agar was cooled at 40 °C and bacteria were inoculated in 1% ratio. After inoculation agar was poured into the petri dishes. Four wells were cut in the Tryptic Soy Agar with a 5-mm cooled flamed cork borer. Feijoa leaves extracts were dissolved with sterile deionized water in the ratio of 1:20 (g/mL) and 20 µL of extracts were inoculated into the three wells, sterile deionized water was used as a control. Plates were incubated at 37 °C for 24 h and the diameter (mm) of inhibition zones was measured and all tests were performed in triplicate.

2.7 Experimental design, modeling and optimization

Response surface methodology

Response surface methodology (RSM) was employed for investigation of the effects of four factors in the total phenolics contents of aqueous feijoa extracts. In this study, RSM was used for the evaluation of four factors on one response. Design-Expert Software (Stat-Ease Inc., Version 11. Minneapolis) was used for RSM. In this study, the factors (independent variables) were solid(feijoa)/solvent(water) ratio (mg/mL, X_1), extraction temperature (°C, X_2), extraction time (min, X_3), and shaking type (vertical or horizontal, X_4) and the response (dependent

variable) was the total phenolic content (TPC, mg/L, Y). The Central Composite Design (CCD) indicating extraction conditions and the responses of TPC are presented in Table 1. The design type and the design model were central composite and reduced cubic, respectively. The data collected were fitted by multiple regressions to obtain a quadratic reduced cubic model and regression coefficients were obtained.

3 Results and discussion

3.1 Response Surface Methodology (RSM)

Experimental design, factors, actual and predicted total phenolic contents obtained by RSM model (central composite design) are presented in Table 1. The reduced cubic model was selected because of the capability to present the best correlation between the dependent variable (total phenolic content) and independent variables (extraction temperature-A, extraction time-B, feijoa leaf concentration-C and shaking type-D) when compared to linear, interactive and quadratic models). Forty experimental runs were performed and details are presented in Table 1. In addition, measured (actual) and predicted TPC are presented in Table 1. The yield of the TPC ranged from 941.06 to 4347.56 mg GAE/L and maximum TPC was observed for the 8th run under the experimental conditions of A= 100 °C; B=5 min; C=100 mg/mL and D=vertical shaking. RSM model statistics are presented in Table 2. As it can be seen, reduced

cubic model provided the most favorable statistical values for modeling of the interrelation between independent variables (factors) and dependent variable (TPC). In other words, the highest adjusted coefficient (R^2_{adj}), predicted coefficient (R^2_{pre}), and the lowest p -value were obtained by reduced cubic model. ANOVA results for linear and combination relationships are presented in Table 2. P -values for linear and combination effects of independent factors on the response (total phenolic content) are presented in Table 2. P -values for extraction temperature (A), feijoa concentration (B) were determined to be lower than 0.0001. P -values less than 0.05 indicate these terms are significant. In this model, the linear effects of A (extraction temperature), B (extraction time), C (feijoa concentration), and D (shaking type) are significant. In addition, the combination effects of AB, AC, AD, BD, A^2 , B^2 , ABD, A^2D , and C^2D are significant. Adjusted and predicted R^2 values of the RSM model were determined to be 0.9759 and 0.9090, respectively. The difference between predicted and adjusted R^2 values is less than 0.2 and this shows that adjusted and predicted R^2 values are in good agreement. The F -value of the model is 84.10 and this implies the model is significant. There is only a 0.01% chance that an F -value this large could occur due to noise. The lack of fit value of the model was found to be 0.0580 and indicating that lack of fit is not significant. This means that the model is extremely accurate without any noise.

The response surface model equation which relate the influence of variables to the total phenolic content is presented by the following

equation (R = total phenolic content, A = extraction temperature, B = extraction time, C = feijoa concentration, D = shaking type):

$$R = 2334.52 + 360.77A - 100.04B + 1042.27C - 97.95D - 80.56AB + 174.72AC + 118.62AD - 66.44BC - 77.56BD + 53.34CD + 326.80A^2 - 237.43B^2 - 73.62C^2 + 104.5ABD + 60.9ACD - 29.65BCD + 166.34A^2D + 114.31B^2D - 137.91C^2D \quad (1)$$

RSM model was developed through 40 runs and residual analysis was performed for evaluation of the response surface model of total phenolic content. The normal plot of residuals, predicted versus actual, residuals versus run, and residual versus predicted are presented in Figure 1A, 1B, 1C, and 1D, respectively. As it can be seen in Figure 1A, favorable correlation was observed between experimental and predicted values since all data dispersed on a straight line. Similarly, the graph of predicted versus actual values are presented in Figure 1B. The actual values are quite close to the predicted ones as perfectly linear correlation were observed between them. The data points are splitted evenly by the 45-degree line, which describes the perfect fit between predicted and actual values.

Figure 1C shows the residuals calculated versus the experimental runs, indicating that there were positive and negative runs. The existence of tendency to have runs of positive and negative residuals is explained by the presence of a certain correlation. This is a plot of the residuals versus the experimental run order. It provides a check for lurking variables that may have influenced

Figure 1. Normal plot of residuals for total phenolic content, TPC. (A) predicted versus actual values, (B) residuals versus experimental run number (C), residuals versus predicted values (D).

Figure 2. Output factor values for maximization of the total phenolic content by response surface methodology design (Desirability=1).

the response during the experiment. The plot should show a random scatter. Trends indicate a time-related variable lurking in the background. Residuals versus predicted plot is presented in Figure 1D; it is used for visual check for the assumption of constant variance. A random scatter with a consistent top to the bottom range of residuals across the predictions was observed on the X1 axis. No residuals were observed outside the red lines of the plot. In other words, no observation was noted that does not fit the RSM model. These results demonstrate the accuracy and precision of the developed RSM model. In addition, output factor values for the maximization of the total phenolic content by the RSM (desirability = 1) are presented in Figure 2. According to the RSM model, extraction temperature, time, concentration, and the shaking type were determined as 100 °C, 5 min, 100 (mg/mL), and vertical shaking, respectively. Most significant ($p < 0.0001$) linear factors were extraction temperature and solid/solvent ratio (mg/mL) of feijoa leaf. As it can be seen, maximum phenolic extraction was obtained at maximum extraction temperature (100 °C). The temperature was reported as a significant factor in terms of extraction of total phenolic content from leaves, an increase in the extraction temperature resulted in a rise in extracted phenolic content (Li et al., 2016). Previous studies reported that extraction temperature was the most important independent variable for the extraction of bioactive compounds from feijoa leaves and increase in the extraction temperature caused positive effect on extraction of bioactive compounds (Poodi et al., 2018). In another study, it was reported that the total phenolic content of extracts increased with increasing temperature (Ballard et al., 2009). Li et al. (2016) showed that

temperature was a significant factor for the extraction of phenolics from perilla leaves and rise was observed in the total phenolic content with rising temperature (Li et al., 2016). Solid/solvent ratio (mg/mL) of feijoa leaf significantly affected the total phenolic extraction ($p < 0.0001$). The maximum phenolic yield was obtained at maximum solid/solvent ratio (100 mg/mL). Previous studies reported that an increase in the solvent concentration might result in higher phenolic yield in the ethanolic or methanolic extracts of some leaves (Silva et al., 2007). However, in our study, aqueous extraction was performed from feijoa leaves and total phenolic yield increased with rising solid/solvent ratio. In a previous research study, bioactive properties of aqueous *Stevia* leaf extracts increased in a dose-dependent manner. In other words, radical scavenging activity raised with increasing *Stevia* leaf concentration in the range of 20-200 µg/mL (Shukla et al., 2012). In our study, p value was found to be lower than 0.005 for the extraction time. Extraction time was selected between 5 and 30 min in the RSM model. However, the maximum phenolic yield was obtained in 5 min (desirability=1). Favorably, shorter extraction times may protect valuable bioactive compounds from deterioration. This result is in agreement with the reported by Poodi et al. (2018) for the extraction of bioactive compounds from feijoa leaves (Poodi et al., 2018). In addition to these results, vertical shaking was determined by RSM for maximum phenolic yield from feijoa leaves. This result may be explained with better penetration of solvent into the feijoa leaves through improved vertical shaking type with upside and downside shaking. Central Composite Design (CCD) of response surface methodology was successfully applied for the optimization of independent variables

to maximize TPC values. Figure 2 presents the desirability value and defined factor values for the most desirable ($D=1.000$) solution for the maximization of total phenolic content. In this study, the most desirable ($D = 1.000$) solution for maximization of TPC corresponds to vertical shaking, extraction time of 5 min, and extraction temperature of 100 °C to obtain maximum TPC value, 4455.44 mg GAE/L.

3.2 Volatile composition of feijoa leaves

Head space/gas chromatography-mass spectrometry analysis was performed for the determination of the volatile composition of dried feijoa leaves. Retention indices and relative abundance percentages of related compounds are presented in Table 3. According to the GC-MS identification results, 20 compounds were detected in feijoa leaf. These compounds were determined by comparing the mass spectra of feijoa leaf to the library of GC-MS. The most abundant volatile components were limonene, linalool, and caryophyllene at the percentages of 33.46%, 18.95% and 22.18%, respectively. Other volatile compounds are α -pinene, β -pinene, myrcene, α -terpinene, (Z)- β -ocimene, (E)- β -ocimene, γ -terpinene, terpineol, α -cubebene, α -copaene, β -bourbonene, α -gurjunene, β -gurjunene, α -humulene, alloaromadendrene, germacrene, and spathulenol. To the best of our knowledge, this is the first attempt for the determination of the volatile composition of feijoa leaves from Turkey. This result is in partially agreement with those reported by Mosbah et al. (2018) about the characterization of feijoa leaves growing in Tunisia. Mosbah et al. (2018) reported that major volatile components were limonene, β -caryophyllene, and aroma dendrene in the feijoa leaves from Tunisia (Mosbah et al., 2018). So far, very little research has been carried out on the subject of the volatile composition of feijoa leaves. In another study, Saj et al. (2008) reported that major volatile compounds were limonene, β -caryophyllene and α -pinene, β -pinene and estragole in the feijoa leaves (Saj et al., 2008). Our results are quite coherent with previous studies and mostly limonene, linalool, and caryophyllene were determined as volatile compounds in the feijoa leaves cultivated in Turkey. According to our results, the most abundant volatile compound was limonene with 33.46% relative abundance ratio. Previous contributions reported that D-limonene showed antioxidant, anticancerogenic, and anti-inflammatory properties with desirable flavor and sensory properties (Sun, 2007). Functional and favorable properties of limonene may contribute to further utilization of feijoa in functional food products in the food sector.

3.3 Phenolic composition of feijoa leaves

Quantitative analyses were performed for the important phenolic standards, which are presented in Table 4. Phenolic composition of the aqueous feijoa leaf extract is presented in Table 4 and Figure 3. The extract of run eight is used for the HPLC analysis. Gallic acid, catechin, syringic acid, ellagic acid, chrysin, caffeic acid, *p*-coumaric acid, ferulic acid, and quercetin were detected as phenolic compounds. Major compounds were catechin, quercetin, and ellagic acid. Our results were quite compatible with previous ones. Up to now, very limited studies were performed for the evaluation of the phenolic content of feijoa. Poodi et al. (2018) reported that major phenolic compounds were

Table 3. Volatile composition of the feijoa leaf from Turkey.

Number	Compound	RI ^a	% content ^b
1	α -pinene	939	0.6 $\pm$ 0.32
2	β -pinene	980	0.5 $\pm$ 0.21
3	myrcene	991	0.9 $\pm$ 0.12
4	α -terpinene	1018	0.3 $\pm$ 0.15
5	limonene^c	1031	33.46 $\pm$ 1.21
6	(Z)- β -ocymene	1040	1.5 $\pm$ 0.84
7	(E)- β -ocymene	1050	0.8 $\pm$ 0.11
8	γ -terpinene	1062	0.7 $\pm$ 0.55
9	linalool	1098	18.95 $\pm$ 1.34
10	terpineol	1189	6.76 $\pm$ 0.82
11	α -cubebene	1347	0.9 $\pm$ 0.14
12	α -copaene	1372	1.02 $\pm$ 0.25
13	β -bourbonene	1384	2.55 $\pm$ 0.63
14	α -gurjunene	1412	1.35 $\pm$ 0.72
15	caryophyllene	1418	22.18 $\pm$ 1.21
16	β -gurjunene	1432	1.5 $\pm$ 0.58
17	α -humulene	1455	1.08 $\pm$ 0.45
18	alloaromadendrene	1463	0.25 $\pm$ 0.13
19	germacrene-d	1576	1.09 $\pm$ 0.44
20	spathulenol	1580	3.6 $\pm$ 0.71
Total Identified			99.9

^aRI: Retention index relative to n-alkanes (C8–C40) on Rtx-5MS fused silica capillary column (30m $\times$ 0.25mm (internal diameter) 0.25 μ m); ^b% Content: The relative compositions of the constituents obtained by GC-MS peak analysis; ^cMost abundant compounds are presented bold.

Table 4. Phenolic composition of the feijoa leaf extract sample with maximum total phenolic content.

N ^o	Bioactive phenolic compounds	Major phenolic content (mg/g)
1	gallic acid	32.25
2	catechin	46.45
3	syringic acid	0.51
4	ellagic acid	41.69
5	chrysin	20.74
6	caffeic acid	12.00
7	<i>p</i> -coumaric acid	2.61
8	ferulic acid	5.40
9	quercetin	42.03

determined as gallic acid, catechin, rutin, ferulic acid, quercetin apigenin, and gallic acid was obtained at the highest concentration by the ultrasound-assisted extraction of feijoa leaf (Tehran, Iran). Common phenolic compounds were gallic acid, catechin, ferulic acid, and quercetin with the current research study. Bimakr et al. (2019) determined the phenolic composition of feijoa leaf extract, which was obtained by modified supercritical carbon dioxide extraction. They reported that the main phenolic compounds were gallic acid, catechin, rutin, ferulic acid, quercetin, and apigenin. Mousavi et al. (2018) reported that feijoa leaves from Iran, included gallic acid, catechin, rutin, ferulic acid, apigenin, and quercetin in their compositions. While the most abundant phenolic compound was gallic acid (98.18 mg/g), least abundant one was apigenin (19.67 mg/g) obtained by supercritical carbon

Figure 3. HPLC chromatogram of the feijoa leaf extract with maximum total phenolic content.

Table 5. Antibacterial activities of *Feijoa sellowiana* leaves extract.

Test microorganisms	Inhibition zone (mm)
Gram positive bacteria	
<i>Bacillus cereus</i> ATCC 11778	17.50 ± 0.09
<i>Bacillus subtilis</i> ATCC 6633	8.33 ± 0.12
<i>Streptococcus mitis</i>	13.83 ± 0.03
<i>Listeria monocytogenes</i> ATCC 13932	15.67 ± 0.06
<i>Staphylococcus aureus</i> ATCC 29213	13.33 ± 0.03
Gram negative bacteria	
<i>Escherichia coli</i> ATCC 25922	14.00 ± 0.10
<i>Salmonella</i> Typhimurium ATCC 14028	13.83 ± 0.08
<i>Klebsiella pneumoniae</i> ATCC 43816	15.00 ± 0.05
<i>Pseudomonas aeruginosa</i> ATCC 27853	13.33 ± 0.03
<i>Escherichia coli</i> O157:H7 ATCC 43888	12.67 ± 0.03
Yeast	
<i>Candida albicans</i> ATCC 10251	12.50 ± 0.05

dioxide extraction (40°C, 150 bar and 60 min) (Mousavi et al., 2018). In another study, Aniq et al. (2019) reported that phenolic composition of the leaves of feijoa mostly included gallic acid, catechin, epicatechin, caffeic acid, and ellagic acid. The most abundant phenolic compound was ellagic acid, according to their UFLC-MS/MS analysis results (Aniq et al., 2019). Our results showed that major phenolic compounds detected in feijoa leaves from Turkey were catechin, quercetin, ellagic acid, and gallic acid with the concentration of 46.45 mg/g, 42.03 mg/g, 41.69 mg/g, and 32.25 mg/g, respectively. In general, similar phenolic compounds were observed in the feijoa leaves of different countries. However, disparities were observed in the quantities of phenolic compounds of different countries. In accordance with the present results, previous studies have demonstrated that phenolic composition of same species may vary related to the regional differences (Ouerghemmi et al., 2016). For example, Ouerghemmi et al. (2016) reported that phenolic composition of *Rosa moschata* showed differences between the Tunisian regions of Nabeul, Bizerte, and Zaghouan. Our findings show that feijoa leaves from Turkey could be used as a potential source

for valuable phenolic compounds such as gallic acid, catechin, quercetin, and ellagic acid.

3.4 Antimicrobial activity of feijoa leaves

Previous reports mostly evaluated the antimicrobial activity of feijoa fruit and feijoa fruit showed antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as fungi (Beyhan et al., 2010; Vuotto et al., 2000). Aqueous feijoa fruit extract showed more antimicrobial activity on Gram-negative bacteria (*Enterobacter aerogenes*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*) when compared to Gram-positive bacteria (Vuotto et al., 2000). To the best of our knowledge, this study is the first effort for the determination of antimicrobial properties of feijoa leaves extract from Turkey. Test microorganisms and inhibition zones are presented in Table 5. Deionized water (control) had no inhibitory effects on the evaluated microorganisms. Antimicrobial activity of feijoa leaf was determined by using agar diffusion method against ten bacteria (5 Gram-positive and 5 Gram-negative) and 1 yeast. Inhibition zones (IZ) varied between 8.33 ± 0.12 mm and 17.50 ± 0.09 mm. As it can be seen from Table 5, *Bacillus cereus* was the most sensitive bacteria with IZ value of 17.50 ± 0.09 mm. Mosbah et al. (2018) evaluated the antimicrobial properties of feijoa leaves extract from Tunisia and they observed lower IZ value for *Bacillus cereus* ATCC 11778. In general our IZ values were higher than the ones determined by Mosbah et al. (2018). However quite similar IZ value was obtained for *Escherichia coli* O157:H7 ATCC 43888 and *Escherichia coli* ATCC35218 as approximately 12.6 mm. In our study, the most resistant bacteria was determined as *Bacillus subtilis* ATCC 6633 with IZ value of 8.33 ± 0.12 mm. In addition, IZ value for *Candida albicans* ATCC 10251 was determined as 12.50 ± 0.05 mm. Mosbah et al. (2018) determined the IZ value as 6.66 ± 0.33 mm for *Candida albicans* ATCC 2019. As it can be seen, IZ variations were observed between results from the feijoa leaves of different countries. This situation may be related to the geographic conditions, which may affect the chemical composition of the feijoa leaves. It was reported that bioactive compounds such as

phenolics and volatile ingredients in the herbs determines the antimicrobial, antioxidant and antifungal properties of related herbs (Tulukcu et al., 2019).

4 Conclusions

In summary, the present study will serve a base for future studies about the evaluation of the phenolic composition, volatile composition, and antimicrobial activity of feijoa leaves from Turkey. In summary, our research developed a RSM model for the extraction of total phenolic compounds from feijoa leaves. Extraction temperature, time, concentration, and the shaking type were determined by the RSM model as 100 °C, 5 min, 100 (mg/mL), and vertical shaking, respectively, for maximization of phenolic compounds. Significant factors were extraction temperature, extraction time, solid/solvent ratio (mg/mL), and shaking type. Most significant ($p < 0.0001$) linear factors were extraction temperature and solid/solvent ratio (mg/mL) of feijoa leaf. In addition, major phenolic compounds were determined as gallic acid, catechin, syringic acid, ellagic acid, chrysin, caffeic acid, *p*-coumaric acid, ferulic acid, and quercetin. In other words, the extract of leaves has high phenolic content with valuable phenolic compounds, which have bioactive properties. In addition, extracts from feijoa leaves showed antimicrobial activity against Gram-positive and Gram-negative bacteria, and one yeast species. Furthermore, volatile profile of feijoa leaf was determined by using GC-MS technique and limonene, linalool and caryophyllene were detected as major volatile compounds. These findings enhance our understanding of further use of feijoa leaves as potential source for valuable bioactive compounds in various industrial products such as dietary supplements, food products (syrops, jams), cosmetic products, etc. Further investigation including high-resolution chromatographic techniques, will provide deeper exploration to discover unknown chemical structures which may have valuable bioactive properties such as antioxidative, anti-inflammatory, antimutagenic, anticarcinogenic, antiangiogenic, antiobesity, hypocholesterolemic, antiatherosclerotic, antidiabetic, antibacterial, and antiviral, anti-aging.

Conflict of interest

The authors declare no competing financial interest.

Author contributions

N. Cebi designed the study, performed the analyses and interpreted the results. O. Sagdic designed the study and interpreted results.

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