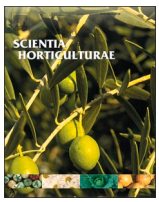
Scientia Horticulturae 313 (2023) 111910

Contents lists available at ScienceDirect

Scientia Horticulturae

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



Insights into the polyphenols extraction from *Actinidia arguta* fruit (kiwiberry): A source of pro-healthy compounds

Catarina Macedo [a], Ana Margarida Silva [a], Ana Sofia Ferreira [a], María de la Luz Cádiz-Gurrea [b], Álvaro Fernández-Ochoa [b], Antonio Segura-Carretero [b], Cristina Delerue-Matos [a], Paulo Costa [c,d], Francisca Rodrigues [a,*]

[a] REQUIMTE/LAQV, ISEP, Polytechnic of Porto, Rua Dr. António Bernardino de Almeida, 4249- 015 Porto, Portugal
[b] Department of Analytical Chemistry, Faculty of Sciences, University of Granada, Spain
[c] UCIBIO – Applied Molecular Biosciences Unit, MedTech-Laboratory of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portugal
[d] Associate Laboratory i4HB - Institute for Health and Bioeconomy, Faculty of Pharmacy, University of Porto, 4050-313 Porto, Portuga

ARTICLE INFO

Keywords:
Actinidia arguta
Ultrasound-assisted extraction
Response surface methodology
Phenolic compounds
Pro-healthy properties

ABSTRACT

Actinidia arguta fruit (also known as kiwiberry) has a remarkable phytochemical profile, being reported as a promising preventive agent/treatment for chronic diseases and a desirable source of bioactive molecules for pharmaceutical and cosmetic industries. Despite these promising indications, investigating the full potential of *A. arguta* fruit remains an unmet need for health applications. This work aims to determine the optimal extraction conditions of antioxidant/antiradical compounds from *A. arguta* fruit (commonly known as kiwiberry), using the Response Surface Methodology (RSM). The effects of probe amplitude (30%-70%), sonication time (5-30 min) and water:ethanol solvent ratio (0%-100%) were assessed. The optimal extraction conditions were achieved using 50% of water, during 17.5 min and using an amplitude of 50%. A total of 22 compounds, including 6 flavonoids and 4 phenolic acids, were identified in the optimal extract through HPLC-ESI-QTOF-MS, which exhibited outstanding antioxidant and antiradical activities (TPC = 18.705 mg GAE/g dw; FRAP = 186.876 μmol FSE/g dw; ABTS = 16.334 mg AAE/g dw; $O_2^{\cdot-}$ IC_{50} = 829.384 μg/mL; HOCl IC_{50} = 16.895 μg/mL; $ROO^{\cdot}$ = 0.18 μg TE/mg dw). Moreover, it displayed antimicrobial activity against *Staphylococcus aureus* (MIC = 32 mg/mL) and *Pseudomonas gingivalis* (MIC = 64 mg/mL) and reduced the growth rate of *Escherichia coli*. *In-vitro* assays demonstrated that the optimal extract successfully decreased the viability of two human carcinoma cell lines, namely TR146 and HSC-3, at 500 μg/mL and above 500 μg/mL, respectively. The present study showed that *A. arguta* fruit is a rich source of compounds with potential to be used for pro-healthy purposes.

1. Introduction

Actinidia arguta, commonly known as kiwiberry, hardy kiwi, baby kiwi or mini kiwi, is a native Asian vine that produces a small kiwi with a smooth and leathery surface, without the typical hair-like fiber that usually covers the exterior of most other species in the genus (Latocha 2017). In addition to its sweet aromatic taste, *A. arguta* fruit presents an interesting phytochemical profile, which provide excellent health benefits (Latocha 2017), despite being an excellent source of antioxidants, carotenoids, chlorophylls, dietary fiber, enzymes, organic acids, minerals and vitamins (Nishiyama et al. 2005; Nishiyama et al. 2008; Wojdyło et al. 2017; Latocha 2017). Remarkably, it contains up to 1301 mg/100 g of fresh weight (fw) of phenolic compounds, 430 mg/100 g fw of vitamin C, 0.93 mg/100 g fw of lutein and 982 mg/100 g fw of myo-inositol (vitamin B8) (Nishiyama et al. 2005; Nishiyama et al. 2008; Wojdyło et al. 2017; Latocha 2017). Among phenolic compounds, the presence of quercetin-3-*O*-rutinoside (49.3 mg/100 g of dry weight (dw) dm), keampferol-3-*O*-galactoside (24.9 mg/100 g dw), neochlorogenic acid (12.9 mg/100 g dw) and cryptochlorogenic acid (9.56 mg/100 g dw) (Wojdyło et al. 2017), compounds with increased importance to avoid or combat diseases in which oxidative stress play a key role (such as cancer, degenerative diseases and diabetes), has been described (Islam 2017; Sosa et al. 2013; Maritim et al. 2003). In fact, early research has pointed out *A. arguta* fruit as a promising preventive

* Correspondence: Francisca Rodrigues, REQUIMTE/LAQV, Polytechnic of Porto - School of Engineering, Rua Dr. António Bernardino de Almeida, 431, 4249-015 Porto, Portugal. Tel.: +351 22 83 40 500; Fax: +351 22 83 21 159
E-mail addresses: francisca.rodrigues@graq.isep.ipp.pt, franciscapintolisboa@gmail.com (F. Rodrigues).

https://doi.org/10.1016/j.scienta.2023.111910
Received 29 December 2022; Received in revised form 2 February 2023; Accepted 3 February 2023
Available online 9 February 2023
0304-4238/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

agent/treatment of various chronic diseases, including some types of cancer, cardiovascular and digestive disorders, with proven anti-allergic, anticholinergic, antidiabetic, anti-inflammatory, anti--hypercholesterolemia, antioxidant, anti-tumor and hepatoprotective activities (Zuo et al. 2012; Yu et al. 2015; Kim et al. 2009a; Gong et al. 2020; Kim et al. 2009b; Zhang et al. 2021).

Notwithstanding, the method applied to extract the compounds of interest from a matrix is of great importance, not only in terms of extraction yield, but also considering its environmental impact. In recent years, different green extraction techniques have emerged, allowing to reduce the energy consumption and employing more ecological alternative solvents, often in smaller quantities (Chemat et al. 2012). Examples of green extraction techniques used for obtain antioxidant compounds from vegetable materials are microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), supercritical fluid extraction (SFE), pulsed electric field (PEF), enzyme assisted extraction and pressurized liquid extraction (PLE) (Panja 2018). Among these, UAE stands out for requiring less time and energy, allowing high extraction yields and maintaining the quality of the extracted compounds (Kumar et al. 2021). Even so, the extraction yield and the compounds functionality are dependent on the parameters used for the extraction, namely the matrix/solvent ratio, time, frequency, power, temperature, amplitude and solvent type (Dzah et al. 2020). Thus, it is crucial to use mathematical and statistical approaches, such as Response Surface Methodology (RSM), to determine the optimal extraction conditions, through the analysis of the interaction between the factors and the response parameters.

The aim of this work is to apply the RSM to optimize the extraction conditions (solvent, time, and amplitude) of kiwiberry by ultrasound-assisted extraction (UAE). The optimal extract was characterized in what concerns to Total Phenolic Content (TPC) and antioxidant/antiradical activities, evaluated by ABTS and FRAP assays. In addition, the radical scavenging capacity, phenolic profile, and antimicrobial activity, along with *in-vitro* effects on oral cell lines, were evaluated for the optimal extract.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical grade and acquired from Sigma-Aldrich (Steinheim, Germany) and Sigma Chemical Co. (St. Louis, USA). Cell culture assay reagents were obtained from Invitrogen Corporation (Life Technologies, S.A., Madrid, Spain).

2.2. Samples

A. arguta (cv. 'Jumbo') fruits were supplied by Mini-Kiwi Farm (Landim, Vila Nova de Famalicão, Portugal). The fruits were dehydrated (Excalibur Food Dehydrator, USA) for 24 h at 40°C and ground in a miller (Solac, professional Mixter 600W, Spain). Afterwards, samples were stored at room temperature (25°C) in the dark until further extraction.

2.3. Ultrasound-assisted extraction (UAE) of A. arguta pomace

The UAE of *A. arguta* fruits, composed by pulp and peel, was performed using an ultrasonic probe processor (Sonics Vibra Cell, model VCX50, Newtown, CT, USA), with a 13 mm probe. The samples were extracted at room temperature, maintaining the 1:40 g/mL sample-to-solvent ratio, 750 W power and 20 kHz frequency. A Response Surface Methodology (RSM), with a Box-Behnken Design (BBD) with 3 levels, 3 independent variables and 17 runs, including 5 center points, was employed to optimize the extraction conditions, namely the probe amplitude, the sonication time and the water:ethanol (H_2O:EtOH) solvent ratio. The amplitude of the probe ranged from 30% to 70%, while the sonication time varied between 5 and 30 min and the ratio water: ethanol ranged from 0% to 100%, in which 0% correspond to 100% EtOH and 100% correspond to 100% H_2O.

After extraction, the samples were filtered through Whatman n° 1 paper and kept at 4°C overnight. Afterwards, they were centrifuged twice at 18 500 × g (13 000 rpm in Megafuge™ 16, Thermo Scientific, Massachusetts, USA) for 10 min at 20°C for debris removal. After centrifugation, the ethanol from ethanolic and hydroethanolic extracts was evaporated and the samples were frozen at -80°C and lyophilized (Cryodos–80, Telstar, Barcelona, Spain). The samples were stored at 4°C until further analysis.

The extraction yield was determined as the ratio between the weight of the dry extract after solvent removal and the weight of dry sample before extraction, as follows:

$$\text{Extraction yield } (\%) = 100 \times \text{ weight of lyophilized extract/weight of dry sample}$$

2.4. Evaluation of total phenolic content (TPC)

The determination of the TPC was performed following the Folin–Ciocalteu procedure designed by Singleton and Rossi (Singleton and Rossi 1965), with minor modifications. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of extract on dry weight (dw) (mg GAE/g dw).

2.5. Evaluation of antioxidant and antiradical activities

2.5.1. Ferric reducing antioxidant power assay (FRAP)

The ferric reducing antioxidant power (FRAP) was accessed as described by Benzie and Strain (Benzie and Strain 1999), with minor modifications. The results were expressed in μmol of ferrous sulphate equivalents (FSE) per gram of extract on dw (μmol FSE/g dw) and in IC_{50} (μg/mL).

2.5.2. $ABTS^{\cdot+}$ radical scavenging activity assay

The extracts $ABTS^{\cdot+}$ scavenging capacity was determined based on the procedure developed by Re *et al.* (Re et al. 1999), with minor modifications. The results were presented as mg ascorbic acid equivalents (AAE) per gram of extract on dw (mg AAE/g dw) and in IC_{50} (μg/mL).

2.5.3. Reactive oxygen species scavenging capacity assays

2.5.3.1. Hypochlorous acid scavenging assay (HOCl). The hypochlorous acid (HOCl) quenching capacity of *A. arguta* optimal extract was determined according to the protocol described by Gomes *et al.* (Gomes et al. 2007), using ascorbic acid as positive control. Results were expressed as the inhibition, in IC_{50} (μg/mL), of HOCl-induced oxidation of DHR to rhodamine.

2.5.3.2. Peroxyl radical scavenging assay ($ROO^{\cdot}$). The peroxyl radical scavenging assay, also known as the oxygen radical absorbance assay (ORAC), was performed for the optimal extract following the procedure developed by Gomes *et al.* (Gomes et al. 2007). Results were expressed as mg TE/g dw.

2.5.3.3. Superoxide anion radical scavenging assay ($O_2^{\cdot-}$). The superoxide anion radical ($O_2^{\cdot-}$) scavenging assay was performed for the optimal extract following the procedure described by Gomes *et al.* (Gomes et al. 2007). The results were presented as the inhibition, in IC_{50} (μg/mL), of the NBT reduction to diformazan.

2.6. Phytochemical characterization of A. arguta pomace by HPLC-ESI-QTOF-MS methodology

The sample was reconstituted at a concentration of 5 mg/mL from the dry extract. This solution was centrifuged, and the supernatant was filtered and aliquoted in a HPLC vial for the HPLC-ESI-QTOF-MS analysis. A chemical standard mix (catechin, epicatechin, chlorogenic and neochlorogenic acids) was prepared at a concentration of 500 mg/L by standard. The mixture of the 4 chemical standards was diluted to the following concentrations for the calibration curves: 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 30 and 50 mg/mL. Three replicates were prepared per calibration dilution as well as for the experimental sample.

The analyses were carried out in a high-pressure liquid chromatography instrument (Agilent 1290 HPLC, Agilent Technologies, Palo Alto, CA, USA) coupled to mass spectrometry with a quadrupole time-of-flight analyzer (Agilent 6545 QTOF Ultra High Definition, Agilent Technologies, Palo Alto, CA, USA). The chromatographic method was based on reverse phase with a C18 column (ACQUITY UPLC BEH, 1.7 μm, 2.1 mm, 150 mm, 130 Å, Waters Corporation), whose column temperature was fixed at 60°C. Water with 0.1% of formic acid and acetonitrile were used as mobile phase A and B, respectively. The following mobile phase gradient was used for optimal separation: 0.00-2.33 min [A:B 99/1], 4.37 min [A:B 93/7], 8.11 min [A:B 86/14], 12.19 min [A:B 76/24], 15.99 min [A:B 60/40], 25.00-28.00 min [A:B 2/98], and 29.00-32.50 min [A:B 99/1]. A mobile phase flow rate of 0.4 mL/min and an injection volume of 5 μL were used. Three replicates were analyzed per calibrant and experimental sample.

MS acquisition was carried out in negative electrospray ionization mode (ESI-) in a mass/charge ratio range between 50 and 1200 m/z. All m/z values were continuously calibrated by means of a continuous infusion of two reference masses: purine (m/z 121.0509) and HP-921 (m/z 922.0098). The experimental samples and the calibrants were analyzed in full scan acquisition mode with a scan rate of 3 spectra/sec. The MS data were acquired in centroid mode. Additionally, three replicates of the experimental sample were analyzed. Other parameters were as follows: gas temperature 200°C; gas flow 10 L/min; nebulizer 20 psig, sheath gas temperature 350°C, sheath gas flow 12 L/min, VCap 4000 V, Nozzle Voltage 500 V. The acquired MS data was converted to a mzML format using MSConverter (Proteowizard) and then processed using the mzMine 2.53 software. The compounds were annotated according to the comparison of the experimental data (Retention time, m/z, molecular formula) with those found in the literature and databases (FoodDB, HMDB, SciFinder, etc.). The limits of detection and quantification were calculated according to a Signal/Noise level of 3 and 10, respectively, according to the IUPAC guidelines.

2.7. Cell viability assays

The HSC-3 and TR146 cell lines were obtained from American Type Culture Collection (ATCC, USA). Passage 9-10 of HSC-3 and passage 26-27 of TR146 were used for the MTT assay. The cells were seeded at a density of 10×10^4 cells/mL and incubated during 24 h at 37°C with supplemented DMEM, in the absence or presence of the optimal extract (concentrations tested from 0.1 to 1000 μg/mL). After this time, the MTT was incubated for 3 h at 37°C. DMEM was used as positive control, while Triton X-100 1% (w/v) was the negative control. The absorbance was measured at 590 nm with background subtraction at 630 nm. Results were expressed as percentages of cell viability.

2.8. Antimicrobial activity screening

The antimicrobial activity was performed using Gram-positive (*Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29922) and Gram-negative strains (*Escherichia coli* ATCC 25992, *Escherichia coli* CTX M2, *Porphyromonas gingivalis* ATCC 33277). The broth microdilution method was used to evaluate the minimum inhibitory concentration (MIC) of all strains, in 96-well plates, according to the Clinical Laboratory Standard Institute (CLSI), with minor modifications (CLSI 2012). The density of each inoculum was adjusted in Mueller Hinton Broth medium (MHB) to 0.08-0.10 (McFarland standard photometric device), and then exposed to the optimal extract in a concentration range of 0 to 64 mg/ml. MICs and growth rates were determined through periodical spectrophotometric readings at 625 nm, 37°C, for 24 h.

2.9. Statistical analysis

The analysis of the data was performed using IBM SPSS Statistics 28.0.1.0 software (SPSS Inc., Chicago, IL, USA). One-way ANOVA was applied to analyze the differences between samples, and post hoc comparison of the means was performed with Tukey's HSD test. A denoting significance was accepted for $p \leq 0.05$. GraphPad Prism 9.2.0 software (La Jolla, CA, USA) was used to determine de area under the curve on the ORAC assay.

3. Results and discussion

3.1. Validation of the experimental design and RSM analysis

To optimize the extraction conditions of the kiwiberry bioactive compounds, a total of 17 experiments were carried out, with the extraction conditions determined by the RSM using the BBD. The parameter values for each experiment and the respective TPC, FRAP and ABTS experimental results are summarized in Table 1.

The selection of a model and the evaluation of its meaningfulness was made by analysis of variance (ANOVA), as detailed in Table 2.

The suggested quadratic model was used for all responses (TPC, FRAP and ABTS). For all cases, the predicted R^2 agrees with the adjusted R^2, as the difference is less than 0.2, and the precision value is higher than 4, indicating an appropriate signal-to-noise ratio and a good adequacy of the model. Moreover, the lack of fit was not significant ($p > 0.05$).

The relation between the independent variables solvent (A), time (B) and amplitude (C) and the dependent variables, namely TPC, FRAP and ABTS, are represented on 3D graphs (Fig. 1).

The analysis of the 3D graphs indicates that among all the varied factors, the solvent seems to be the one that leads to the greatest variation in the response level. Since more than two factors are being studied, the factors that most affect each response can be identified more accurately by the analysis of the perturbation plot. The perturbation graphs generated by the RSM represents the deviation of the response from the center point (50% H_2O, 17.5 min, 50% amplitude) of the experimental regions, as shown in Fig. 2.

The curvature in the solvent factor (A) for TPC, FRAP and ABTS demonstrates that the response is particularly sensitive to variations in this factor.

The relative impact of solvent (A), time (B) and amplitude (C) factors on TPC, FRAP and ABTS is summarized in the following coded equations:

$$\mathbf{TPC} = 21.95 + 2.22A - 0.3762B + 0.3318C + 0.1783AB + 0.8658AC - 1.41BC - 9.47A^2 - 1.42B^2 - 0.6724C^2$$

$$\mathbf{FRAP} = 203.96 + 20.70A - 5.16B - 14.93C - 2.29AB - 20.04AC + 3.84BC - 113.67A^2 - 11.63B^2 + 7.65C^2$$

$$\mathbf{ABTS} = 18.69 + 1.99A + 0.2383B + 0.0961C - 0.2355AB + 0.1207AC - 1.22BC - 13.04A^2 - 0.9984B^2 - 1.40C^2$$

However, if only the factors with $p \leq 0.05$ (Table 2) are considered, it is possible to verify that the independent variable solvent (A) was the only

Table 1
Extraction conditions determined by Response Surface Methodology (RSM) with Box-Behnken Design (BBD) and the respective experimental response values. Response values and yield are expressed as mean ± standard deviation (n = 3).

Run	Independent variables			Response values			Yield (%)
	A, Solvent (%H_2O)	B, Time (min)	C, Amplitude (%)	TPC (mg GAE/g DW)	FRAP (μmol FSE/g DW)	ABTS (mg AAE/g DW)	
1	0	17.5	30	10.499 ± 0.731	67.546 ± 2.219	2.171 ± 0.518	33.6 ± 3.2
2	50	30	70	17.882 ± 1.267	177.659 ± 4.344	15.694 ± 2.856	56.16 ± 7.5
3	100	17.5	70	14.834 ± 0.497	88.241 ± 3.592	6.550 ± 0.331	54.9 ± 6.0
4	50	5	30	18.987 ± 0.235	229.976 ± 4.203	14.439 ± 1.414	51.9 ± 5.7
5	100	17.5	30	13.064 ± 0.196	160.167 ± 2.826	6.554 ± 1.180	55.1 ± 3.5
6	50	5	70	23.105 ± 1.901	194.417 ± 5.470	17.500 ± 1.543	48.5 ± 4.5
7	50	17.5	50	24.071 ± 0.949	218.047 ± 3.670	21.635 ± 2.763	51.8 ± 7.2
8	50	17.5	50	20.543 ± 0.968	195.650 ± 7.123	18.175 ± 1.189	50.6 ± 8.0
9	50	17.5	50	21.439 ± 0.812	191.805 ± 5.592	17.635 ± 1.534	61.6 ± 11.6
10	0	5	50	8.498 ± 0.609	59.348 ± 3.376	2.574 ± 0.649	27.5 ± 2.8
11	100	30	50	13.955 ± 0.793	93.400 ± 1.819	6.247 ± 1.224	52.1 ± 3.6
12	50	17.5	50	23.697 ± 1.332	204.640 ± 17.734	17.992 ± 1.055	61.5 ± 10.1
13	0	30	50	9.030 ± 1.001	67.721 ± 6.607	3.373 ± 0.663	44.1 ± 4.2
14	50	17.5	50	19.983 ± 0.983	209.648 ± 4.823	17.988 ± 1.721	58.4 ± 7.1
15	100	5	50	12.710 ± 0.607	94.174 ± 2.986	6.390 ± 0.763	50.2 ± 7.3
16	50	30	30	19.423 ± 2.718	197,849 ± 6,567	17.495 ± 1.332	62.1 ± 9.8
17	0	17.5	70	8.806 ± 0.540	75,783 ± 1,7694	1.684 ± 0.906	39.6 ± 3.9

Table 2
Quadratic model summary and analysis of variance (ANOVA) of TPC (mg GAE/g DW), FRAP (μmol FSE/g DW) and ABTS (mg AAE/g DW) of kiwiberry extracts, and model summary statistics.

Source	Sum of squares			Mean squares			F-value			*p*-value*		
	TPC	FRAP	ABTS	TPC	FRAP	ABTS	TPC	FRAP	ABTS	TPC	FRAP	ABTS
Quadratic Model	452.85	62824.17	786.70	50.32	6980.46	87.41	17.73	44.30	49.84	0.0005	<0.0001	<0.0001
A-Solvent	39.29	3427.27	31.76	39.29	3427.27	31.76	13.85	21.75	18.11	0.0074	0.0023	0.0038
B-Time	1.13	213.06	0.4541	1.13	213.06	0.4541	0.3991	1.35	0.2589	0.5476	0.2830	0.6265
C-Amplitude	0.8805	1783.20	0.0739	0.8805	1783.20	0.0739	0.3103	11.32	0.0421	0.5949	0.0120	0.8432
AB	0.1271	20.92	0.2218	0.1271	20.92	0.2218	0.0448	0.1327	0.1265	0.8384	0.7264	0.7326
AC	3.00	1606.53	0.0583	3.00	1606.53	0.0583	1.06	10.19	0.0333	0.3382	0.0152	0.8605
BC	8.01	59.05	5.91	8.01	59.05	5.91	2.82	0.3747	3.37	0.1369	0.5598	0.1090
A^2	377.88	54402.68	716.03	377.88	54402.68	716.03	133.16	345.23	408.26	<0.0001	<0.0001	<0.0001
B^2	8.55	569.33	4.20	8.55	569.33	4.20	3.01	3.61	2.39	0.1262	0.0991	0.1658
C^2	1.90	246.11	8.31	1.90	246.11	8.31	0.6709	1.56	4.74	0.4397	0.2516	0.0660
Residual	19.86	1103.08	12.28	2.84	157.58	1.75						
Lack of Fit	6.20	655.03	1.25	2.07	218.34	0.4153	0.6055	1.95	0.1506	0.6454	0.2636	0.9241
Pure Error	13.66	448.04	11.03	3.42	112.01	2.76						
Cor Total	472.72	63927.25	798.98									
	TPC	**FRAP**	**ABTS**									
Predicted R^2	0.7449	0.8251	0.9535									
Adjusted R^2	0.9040	0.9606	0.9649									
Adequate precision	10.5994	17.5344	16.2497									

* Significant at p≤0.05

factor that influenced the TPC and ABTS responses, while the solvent (A) and the amplitude (C) influence the FRAP response values. Thus, the proposed equation to predict the TPC, FRAP and ABTS from the remaining system parameters are:

$$\mathbf{TPC} = 21.95 + 2.22A - 9.47A^2$$

$$\mathbf{FRAP} = 203.96 + 20.70A - 14.93C - 20.04AC - 113.67A^2$$

$$\mathbf{ABTS} = 18.69 + 1.99A - 13.04A^2$$

According to the desirability index (Fig. 3), the optimal extraction conditions to maximize the antioxidant/antiradical activity and the TPC were 50% H_2O, during 17.5 min, using an amplitude of 50% (R^2 = 0.865133), with predicted results of 21.947 mg GAE/g dw for TPC, 203.958 μmol FSE/g dw for FRAP and 18.685 mg AAE/g dw for ABTS.

The optimal extraction conditions were confirmed by comparing the predicted and experimental values obtained (Table 3).

The experimental values for the 3 responses were similar to the predicted ones (p>0.05), allowing the validation of the experimental design and demonstrating the meaningfulness of the model.

The optimal extract also achieved an excellent extraction yield (56.4%). Further studies were carried out using the optimal extract aiming to characterize its phytochemical profile and pro-healthy properties.

3.2. Phytochemical profile of optimal kiwiberry extract

The phytochemical profile of the kiwiberry optimal extract was analyzed by LC-MS, resulting in a total of 22 annotated compounds. Table 4 summarizes all information regarding the retention time (RT), m/z ratio, error (in ppm), molecular formula, and name of each proposed compound.

As can be observed, a total of six flavonoids were identified, namely catechin, epicatechin, isoquercitrin, quercitrin, quercetin and luteolin. These secondary metabolites are produced by numerous plant species, and have been associated with different biological effects, such as anti-cancer, anti-inflammatory, and anti-allergic activities (Kopustinskiene et al. 2020; Kawai et al. 2007). While other studies reported the presence of rutin, quercetin-3-*O*-glucoside, acetyl glycoside and luteolin hexoside in kiwiberry pulp and fruit extracts using HPLC-DAD-MS and

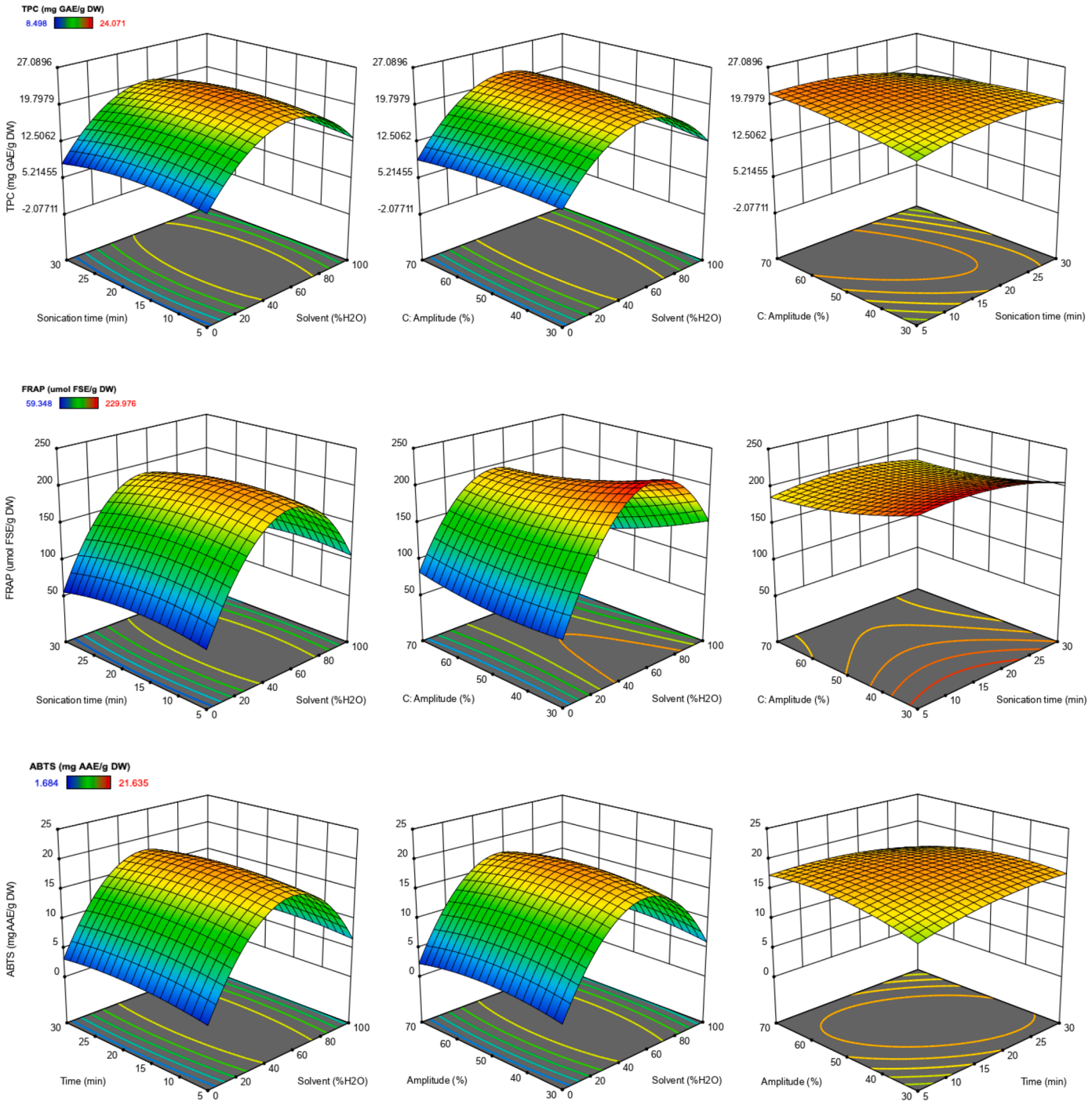


Fig. 1. Three-dimensional (3D) representation of the effect of the independent variables (solvent, time, and amplitude) on the dependent variables (TPC, FRAP and ABTS) of kiwiberry extracts obtained by UAE.

HPLC-DAD-ESI-MS, its presence was not detected in this study (Pinto et al. 2021; Silva et al. 2019). Twelve organic acids were identified, of which 5 are phenolic acids (coumaroyl-hydroxycitric acid, vanillic acid glucoside, neochlorogenic acid, chlorogenic acid and caffeoyl glucose), ubiquitously found in plants and that have well reported biological effects such as antimicrobial, anticancer, anti-inflammatory or anti-mutagenic activities (Kumar and Goel 2019).

In 2021, Błaszczak *et al.* reported the qualitative phenolic composition of kiwiberry (cv. 'Weiki') through micro-HPLC-QTRAP-MS/MS, after extraction by high-pressure processing (HPP) (Błaszczak et al. 2021). According to the authors, caffeic acid, *p*-coumaric acid, 4-hydroxybenzoic acid, chlorogenic acid, caftaric acid and ferulic acid were identified in the fresh fruit as well as in the HPP extracts. More recently, Zhang *et al.* (2022) identified, by UPLC-Q-TOF-MS^{E}, 63 compounds in *A. arguta* fruits, among flavonols, phenolic acids, flavan-3-ols, anthocyanins, and their derivatives. Glycosylated forms of kaempferol and quercetin were the principal phenolic compounds identified, while coumaroyl- or caffeoyl-type phenolic acid were also present (Zhang et al. 2022). Likewise, three new succinate-phenolic conjugates were identified in kiwiberry, namely argutinosides J–L in the last year (Ahn et al., 2022). Finally, Shen *et al.* (2022) reported the high content of kiwiberry in glycosides, namely isopropyl apiosylglucoside, 6-*O*-acetylarbutin, benzyl gentiobioside, zizybeoside I, hydroxytyrosol 4-*O*-glucoside, phenethyl rutinoside. These compounds are responsible

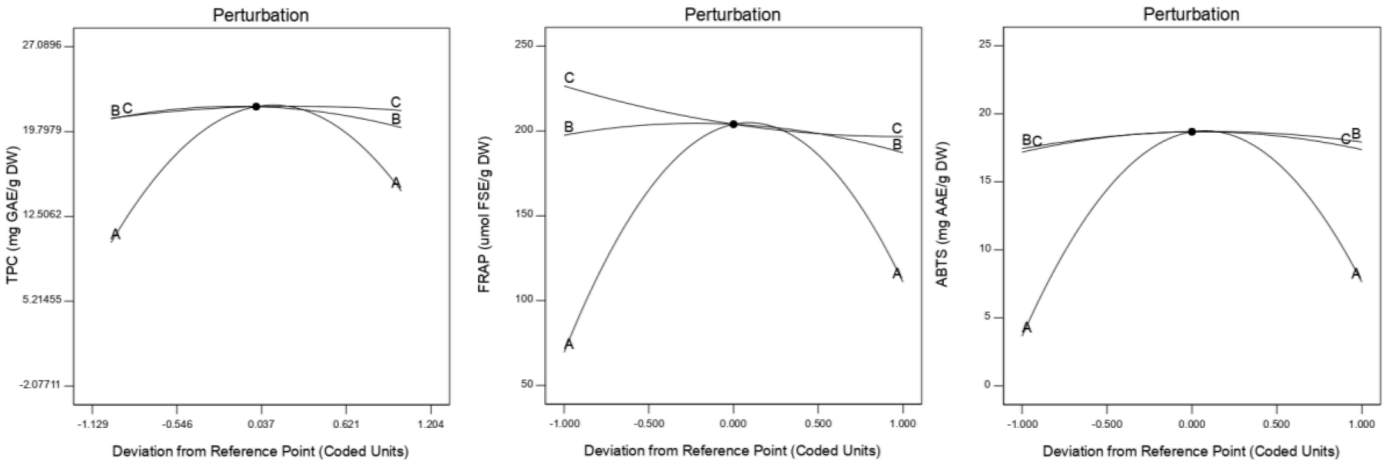


Fig. 2. Perturbation plots of TPC, FRAP and ABTS caused by solvent (A), time (B) and temperature (C), generated by the Design-Expert program.

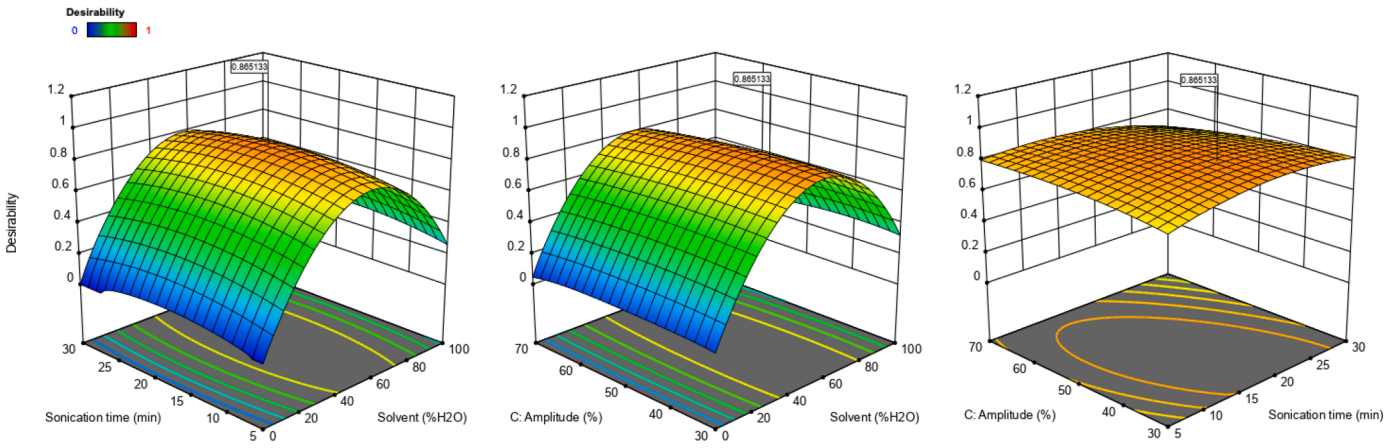


Fig. 3. Desirability index and perturbation graphs for the combined responses of kiwiberry optimal extract obtained by UAE.

Table 3
TPC, FRAP and ABTS results of the optimized extract of kiwiberry. Results are expressed as mean ± standard deviation (n = 3). $p \leq 0.05$ indicates significant differences.

	TPC (mg GAE/g dw)	FRAP (μmol FSE/g dw)	ABTS (mg AAE/g dw)
Experimental value	18.705 ± 0.551	186.876 ± 1.546	16.334 ± 0.832
Predicted value	21.947 ± 1.685	203.958 ± 12.553	18.685 ± 1.324
***p*-value**	0.112	0.121	0.128

for the characteristic fruit aroma. To the best of our knowledge, the present work is the first study that identified coumaroyl-hydroxycitric acid and vanillic acid glucoside in kiwiberry. In addition, four compounds identified in kiwiberry extract were probably responsible for the observed antioxidant/antiradical properties, namely chlorogenic acid (0.217 μg/mg dw), neochlorogenic acid (0.0528 μg/mg dw), catechin (0.0060 μg/mg dw) and epicatechin (0.0024 μg/mg dw) (Table 5).

These results are in agreement with the ones obtained by Zhang *et al.* that also identified chlorogenic acid as one of the predominant phenolic acids in *A. arguta* fruits, ranging from 14.2 to 37.4 μg/g fw in the flesh and between 60.4 and 99.3 μg/g fw in the peel (Zhang et al. 2021).

3.3. Evaluation of antioxidant and antiradical activities

The TPC as well as the antioxidant and antiradical capacities using FRAP and ABTS assays, respectively, were assessed for the kiwiberry optimal extract (Table 3 and 4). The TPC achieved a value of 18.705 mg GAE/g dw (Table 3), which is higher than that the one obtained by Pinto *et al.* using freeze-dried *A. arguta* pulp (12.21 mg GAE/g dw) (Pinto et al. 2021). According to different studies (Latocha et al. 2015; Zhang et al. 2021), the kiwiberry peel is up to 12.6-fold richer in phenolic compounds than the flesh, which indicates that the inclusion of the skin in this study may have been the differentiating factor when compared to Pinto *et al.* Regarding the FRAP assay, the values obtained were 186.876 μmol FSE/g dw (Table 3), with an IC_{50} = 168.190 μg/mL (Table 4). Identically to the TPC, these results are also higher than the ones obtained by Pinto *et al.* (151.41 μmol FSE/g dw) (Pinto et al. 2021). The kiwiberry optimal extract also showed an effective $ABTS^{\cdot+}$ scavenging activity, achieving a value of 16.334 mg AAE/g dw (Table 3), which agrees with the IC_{50} = 2152.816 mg/mL (Table 4) obtained, considering the ascorbic acid positive control used (IC_{50} = 34.805 mg/mL). Noteworthy, most of the studies present the results in fresh weight, being difficult to compare with the results obtained in the present work (Pliszka et al. 2016; Wang et al. 2018; Xiangxue et al. 2016; Jeong et al. 2020).

Regarding the radical scavenging capacity of oxygen species, the optimal extract presented an IC_{50} = 829.384 μg/mL (Table 6) for the $O_2^{\cdot-}$, a result that is considerably lower than the one reported by Pinto *et al.* (IC_{50} = 50.59 μg/mL) using freeze-dried *A. arguta* pulp (Pinto et al. 2021). Nevertheless, this value is considerably better than the one reported by Silva *et al.* for infusions and decoctions of *A. arguta* fruits, since the authors did not obtained an IC_{50} at the highest concentration tested (1 g/mL) (Silva et al. 2019). The IC_{50} obtained for HOCl was 16.895

Table 4
Chemical characterization and quantification of the principal phytochemical compounds of A. arguta extract determined by HPLC-ESI-QTOF-MS.

Proposed compound	Molecular formula	RT	Observed m/z	Theoretical m/z	Error (ppm)
Flavonoids					
Catechin	C15H14O6	8.53	289.0719	290.0796	-3.4
Epicatechin	C15H14O6	9.76	289.0718	290.0796	-3.4
Isoquercitrin	C21H20O12	13.50	463.0890	464.0960	-2.1
Quercitrin	C21H20O11	14.25	447.0938	448.1011	-2.2
Quercetin	C15H10O7	17.27	301.0345	302.0432	-3.3
Luteolin	C15H10O6	18.86	285.0408	286.0483	-3.5
Organic acids					
Malic acid	C4H6O5	1.22	133.0160	134.0221	-7.5
Quinic acid	C7H12O6	1.12	191.0553	192.0639	-5.2
Citric acid	C6H8O7	1.48	191.0199	192.0276	-5.2
Oxoadipic acid	C6H8O5	2.65	159.0293	160.0377	-6.3
Methyl malonate	C5H8O4	2.93	131.0341	132.0428	-7.6
Furoic acid	C5H4O3	5.12	111.0033	112.0166	-9.0
Dimethyl citrate	C8H12O7	5.25	219.0509	220.0589	-4.5
Phenolic acids					
Coumaroyl-hydroxycitric acid	C15H14O10	1.38	353.0534	354.0592	-2.8
Vanillic acid glucoside	C14H18O9	2.63	329.0873	330.0956	-3.0
Neochlorogenic acid	C16H18O9	6.95	353.0884	354.0956	-2.8
Caffeoyl glucose	C15H18O9	8.24	341.0885	342.0956	-2.9
Chlorogenic acid	C16H18O9	9.14	353.0873	354.0956	-2.8
Others					
Glucose	C6H12O6	1.00	179.0553	180.0639	-5.6
Glucovanillin	C14H18O8	5.50	313.0939	314.1007	-3.2
Isopentyl gentiobioside	C17H32O11	6.47	411.1882	412.1950	-2.4
Aucuboside	C15H22O9	6.65	345.1188	346.1269	-2.9

μg/mL, which is slightly higher than the value reported by Pinto *et al.* (Pinto et al. 2021) using freeze-dried *A. arguta* pulp (IC_{50} = 12.77 μg/mL). Silva *et al.* (Silva et al. 2019) obtained a similar value for the *A. arguta* fruit decoctions (IC_{50} = 15.50 μg/mL). In contrast, the IC_{50} value obtained in the present study is lower than the one reported by Silva *et al.* (Silva et al. 2019) using infusions of *A. arguta* fruits (IC_{50} = 27.49 μg/mL). The $ROO^{\cdot}$ scavenging capacity of the optimal extract was 0.18 μg TE/mg dw. To the best of our knowledge, there is no other studies that evaluated the scavenging capacity against this oxygen radical species.

3.4. Antimicrobial activity assessment

Five bacterial strains that may pose a risk to human health were selected for the antimicrobial assays, namely *S. aureus* ATCC 25923, *E. faecalis* ATCC 29922, *E. coli* ATCC 25992, *E. coli* CTX M2 and *P. gingivalis* ATCC 33277. *S. aureus* is a gram-positive bacteria that cause a wide variety of clinical diseases, such as pneumonia, endocarditis, and bacteremia (Chang et al. 2020); *E. faecalis* is a commensal organism of the gut microbiota that has emerged as a multidrug resistant pathogen (Van Tyne et al. 2013); *E. coli* is a commensal commonly found in the lower intestine of different organisms, including humans, being capable of causing infections in extraintestinal environments; while *E. coli* CTX M2 is a β-lactamase producer strain that provides multi-resistance to beta-lactam antibiotics (*e.g.* penicillin, cephamycins and cephamycins); *P. gingivalis* is one of the main pathogenic bacteria in periodontitis, and a potential mediator of a wide variety of chronic inflammatory diseases, such as diabetes, orodigestive cancer and rheumatoid arthritis (Kebschull et al. 2010; Zhou and Luo 2019; Ishikawa et al. 2013; Perricone et al. 2019). Table 7 summarize the Minimum Inhibitory Concentration (MIC) and growing rates of the optimal extract. As it is possible to observe, no MIC was detected for *E. coli* ATCC 25992, *E. coli* CTX M2 and *E. faecalis* ATCC 29922 at the highest tested concentration (64 mg/mL). Nonetheless, there was a slowdown in the growth rate of *E. coli* ATCC 25992 in the presence of the *A. arguta* optimal extract. In addition, a MIC was detected for *S. aureus* ATCC 25923 (32 mg/mL) and for *P. gingivalis* ATCC 33277 (64 mg/mL). These results indicate that the optimal extract can potentially be used to prevent the proliferation of some bacterial strains that could cause severe complications.

3.5. Cell viability assays

Two buccal cell lines, namely TR146 and HSC-3, were employed to evaluate the cell viability effects after exposure to the optimal extract. These cell lines were selected considering the oral exposure to the fruit. TR146 is a cell line derived from human squamous cell carcinoma that mimics the normal human buccal epithelium, while HSC-3 is a human tongue squamous carcinoma cell line (Jacobsen et al. 1999; Lin et al. 2020; Erdem et al. 2007). Fig. 4 summarizes the obtained results.

The exposure of TR146 to increasing concentrations (125 – 2000 μg/mL) of *A. arguta* optimal extract did not significantly decrease the cell viability ($p>0.05$), except for the concentration of 500 μg/mL that led to a viability of 86.20%. Regarding the HSC-3 cell line, after 24 h of incubation in the presence of different optimal extract concentrations (125 – 2000 μg/mL) it was possible to observe a decrease of the viability at the highest concentrations tested. No significant differences ($p>0.05$) were noted between concentrations of 125 μg/mL (105.85%) and 250 μg/mL (91.92%); however, there were differences ($p\leq0.05$) between these concentrations and the highest concentrations tested, namely 500 μg/mL (69.14%), 1000 μg/mL (62,29%) and 2000 μg/mL (56.33%). This decrease in the viability of HSC-3 cells incubated with antioxidant-rich extracts has already been verified in other studies using red fruit

Table 6
Antioxidant/antiradical activities of the A. arguta optimal extract. Results are expressed as mean ± standard deviation (n = 3).

			Reactive oxygen species (ROS)		
	FRAP IC_{50} (μg/mL)	ABTS IC_{50} (mg/mL)	$O_2^{\cdot-}$ IC_{50} (μg/mL)	HOCl IC_{50} (μg/mL)	$ROO^{\cdot}$ μg TE/mg dw
Optimized extract	168.190 ± 8.701	2152.816 ± 41.730	829.384 ± 47.734	16.895 ± 2.880	0.18 ± 0.005
Positive controls					
Gallic acid	-	-	32.211 ± 1.503	-	-
Ascorbic acid	-	34.805 ± 0.263	-	3.154 ± 0.381	1.778 ± 0.416

Table 5
Quantification of the principal phytochemical compounds in kiwiberry optimal extract.

Standard	LOD (μg/mL)	LOQ (μg/mL)	Calibration range (μg/mL)	Calibration equation	R^2	Quantification (μg/mg dw)
Chlorogenic acid	0.0026 ± 0.0006	0.009 ± 0.002	0.05-5	y = 995885x + 99149	0.9982	0.217 ± 0.006
Neochlorogenic acid	0.0022 ± 0.0005	0.007 ± 0.002	0.005-1	y = 2000000x + 448.6	0.9999	0.0528 ± 0.0009
Catechin	0.0027 ± 0.0006	0.009 ± 0.002	0.005-1	y = 1000000x + 6014.7	0.9997	0.0060 ± 0.0008
Epicatechin	0.0014 ± 0.0002	0.0046 ± 0.0007	0.01-1	y = 2000000x + 7044	0.9999	0.0024 ± 0.0006

Table 7
Minimum Inhibitory Concentration (MIC) and growing rates of A. arguta optimal extract on the bacterial strains tested. Results are expressed as mean ± standard deviation (n = 3).

Concentration (mg/mL)	Growing rate/min^{-1}				
	S. aureus ATCC 25923	*E. coli* ATCC 25992	*E. coli* CTX M2	*E. faecalis* ATCC 29922	*P. gingivalis* ATCC 33277
0	0.1065 ± 0.0023	0.1325 ± 0.0124	0.0630 ± 0.0011	0.0845 ± 0.0005	0.1820 ± 0.0012
4	0.0511 ± 0.0085	0.0712 ± 0.0016	0.0211 ± 0.0019	0.0456 ± 0.0009	0.0310 ± 0.0043
8	0.0405 ± 0.0060	0.0843 ± 0.0032	0.0329 ± 0.0025	0.0539 ± 0.0039	0.0205 ± 0.0012
16	0.1126 ± 0.0097	0.0803 ± 0.0025	0.0220 ± 0.033	0.0402 ± 0.0027	0.0273 ± 0.041
32	MIC	0.0591 ± 0.0041	0.0290 ± 0.0016	0.1471 ± 0.0196	0.0115 ± 0.0022
64	No growth	0.0578 ± 0.0034	0.0705 ± 0.0009	0.0645 ± 0.057	MIC

(*Pandanus conoideus Lam*) ethyl acetate extract (Rahmawati et al. 2021), areca nut (*Areca catechu*) ethanolic extract (Sari et al. 2018), andrographis (*Andrographis paniculate*) methanolic extract (Suzuki et al. 2016), and pomegranate (*Punica granatum*) aqueous extract (Peng et al. 2020). According to the authors, this effect is due to the induction of apoptosis through intrinsic and extrinsic pathways. The incubation with areca nut extract increased the caspase-3 activation, the absence of which can lead to cancer cell survival (Sari et al. 2018), while red fruit extract induced apoptosis through the involvement of caspase-8, Bid, cytochrome C, and caspase-3 (Rahmawati et al. 2021). Further studies are needed to identify the metabolic pathways enrolled in the cell viability decrease after exposure to kiwiberry optimal extract, being probably the phenolic compounds, well known by their anticancer effect, responsible for this viability decrease.

4. Conclusion

There is an undeniable need to prevent diseases that affect millions of people around the world, such as cancer and degenerative diseases. In this study, the potential of kiwiberry as source of pro-healthy compounds was assessed. A mathematical model was applied to optimize the bioactive compounds extraction, achieving the optimal extraction conditions with 50% H_2O, during 17.5 min, using an amplitude of 50%. For the optimal extract, a total of 22 compounds were identified by HPLC-ESI-QTOF-MS, including 6 flavonoids and 4 phenolic acids. The *A. arguta* optimal extract displayed remarkable antioxidant and antiradical activities, as well as antimicrobial activity against *S. aureus* and *P. gingivalis*. Moreover, the extract decreased the cell viability of two human squamous cell carcinoma cell lines (TR146 and HSC-3). Further research is required to fully understand the cell molecular effects of the biologically active compounds present on kiwiberry extract, despite the indications that it could be used for healthy purposes.

Funding

This work received financial support from project EXPL/BAA-GR/0663/2021 – Kiwi4Health –Exploring the Eco-Innovative Re-Use of Kiwiberry, supported by national funds by FCT/MCTES and by the projects UIDB/50006/2020 and UIDP/50006/2020 through national funds. This work was also financed by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., in the scope of the project UIDP/04378/2020 and UIDB/04378/2020 of the Research Unit on Applied Molecular Biosciences - UCIBIO and the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions Statement

Catarina Macedo, Ana Margarida Silva, Ana Sofia Ferreira, Maria de la Luz Cádiz-Gurrea, Álvaro Fernandez-Ochoa, Antonio Segura-Carretero and Francisca Rodrigues performed the investigation. Francisca Rodrigues, Antonio Segura-Carretero and Cristina Delerue-Matos supervised the work; Francisca Rodrigues, Antonio Segura-Carretero and Cristina Delerue-Matos supervised the project; Francisca Rodrigues, Antonio Segura-Carretero and Cristina Delerue-Matos obtained the project funding; Catarina Macedo, Ana Margarida Silva, Ana Sofia Ferreira, Maria de la Luz Cádiz-Gurrea, Álvaro Fernandez-Ochoa, Antonio

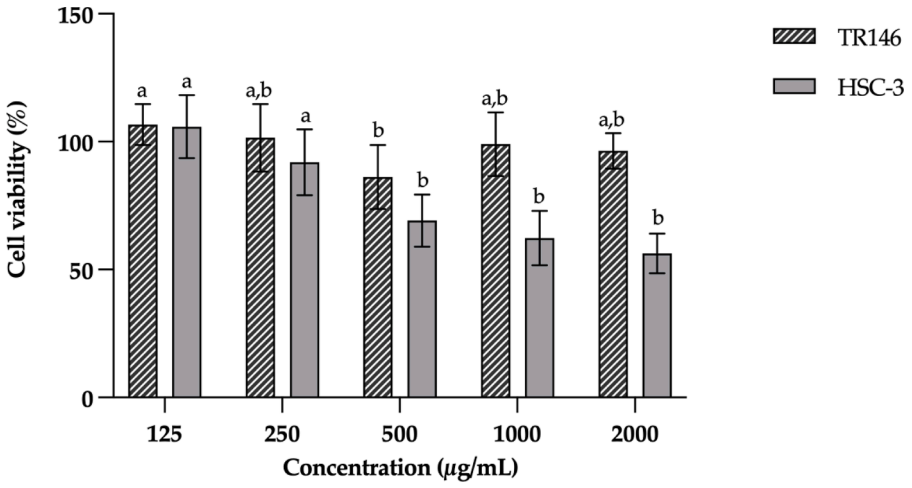


Fig. 4. Effects of *A. arguta* optimal extract exposure on the viability of TR146 and HSC-3 cells at different concentrations (125-2000 μg/mL), measured by MTT assay. Values are expressed as mean ± standard deviation ($n = 3$). Different letters represent significant differences on cell viability between different concentrations on the same cell line ($p \leq 0.05$), according to Tukey's HSD test.

Segura-Carretero and Francisca Rodrigues wrote the main manuscript; Catarina Macedo, Ana Margarida Silva, Ana Sofia Ferreira, Maria de la Luz Cádiz-Gurrea, Álvaro Fernandez-Ochoa, Antonio Segura-Carretero, Francisca Rodrigues and Cristina Delerue-Matos edited the manuscript.

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.

Data availability

The authors do not have permission to share data.

Acknowledgments

The authors kindly thanks Mini-Kiwi Farm (Landim, Vila Nova de Famalicão, Portugal) for the samples. Catarina Macedo, Ana Margarida Silva and Ana Sofia Ferreira are thankful for their Ph.D. grants (2022.12642.BD, SFRH/BD/144994/2019 and SFRH/BD/7519/2020, respectively), financed by POPH-QREN and subsidized by the European Science Foundation and Ministério da Ciência, Tecnologia e Ensino Superior. Francisca Rodrigues (CEECIND/01886/2020) is thankful for her contract financed by FCT/MCTES—CEEC Individual Program Contract. Antonio Segura Carretero is thankful to the Spanish Ministry of Education and Vocational Training for the Senior professors and researchers international stay grant (PRX21/00403). The authors María de la Luz Cádiz Gurrea and Álvaro Fernández Ochoa would like to thank the Regional Ministry of Economy, Knowledge, Enterprise and Universities of Andalusia for the contract for Young Researchers (PAIDI) at the University of Granada.

References

Ahn, J.H., Yeon, S.W., Ryu, S.H., Lee, S., Turk, A., Hwang, B.Y., Lee, M.K., 2022. Three new succinate-phenolic conjugates from the fruits of *Actinidia arguta*. Phytochem. Letters 48, 128–131. https://doi.org/10.1016/j.phytol.2022.01.019.

Błaszczak, W., Latocha, P., Jeża, M., Wiczkowski, W., 2021. The impact of high-pressure processing on the polyphenol profile and anti-glycaemic, anti-hypertensive and anti-cholinergic activities of extracts obtained from kiwiberry (*Actinidia arguta*) fruits. Food. Chem. 343, 128421 https://doi.org/10.1016/j.foodchem.2020.128421.

Benzie, I.F.F., Strain, J.J., 1999. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 15–27. https://doi.org/10.1016/S0076-6879(99)99005-5.

Chang, J., Lee, R.E., Lee, W., 2020. A pursuit of *Staphylococcus aureus* continues: a role of persister cells. Arch. Pharm. Res. 43, 630–638. https://doi.org/10.1007/s12272-020-01246-x.

Chemat, F., Vian, M.A., Cravotto, G., 2012. Green extraction of natural products: concept and principles. Int. J. Mol. Sci. 13, 8615–8627. https://doi.org/10.3390/ijms13078615.

CLSI, 2012. Methods for Dilution Antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard—Ninth Edition. CLSI document, pp. M07–MA9.

Dzah, C.S., Duan, Y., Zhang, H., Wen, C., Zhang, J., Chen, G., Ma, H., 2020. The effects of ultrasound assisted extraction on yield, antioxidant, anticancer and antimicrobial activity of polyphenol extracts: A review. Food Biosci 35, 100547. https://doi.org/10.1016/j.fbio.2020.100547.

Erdem, N.F., Carlson, E.R., Gerard, D.A., Ichiki, A.T., 2007. Characterization of 3 oral squamous cell carcinoma cell lines with different invasion and/or metastatic potentials. J. Maxillofac. Surg. 65, 1725–1733. https://doi.org/10.1016/j.joms.2006.11.034.

Gomes, A., Fernandes, E., Silva, A.M.S., Santos, C.M.M., Pinto, D.C.G.A., Cavaleiro, J.A. S., Lima, J.L.F.C., 2007. 2-Styrylchromones: Novel strong scavengers of reactive oxygen and nitrogen species. Bioorg. Med. Chem. 15, 6027–6036. https://doi.org/10.1016/j.bmc.2007.06.046.

Gong, D.S., Sharma, K., Kang, K.W., Kim, D.W., Oak, M.H., 2020. Endothelium-dependent relaxation effects of *Actinidia arguta* extracts in coronary artery: Involvement of eNOS/Akt pathway. J. Nanosci. Nanotechnol. 20, 5381–5384. https://doi.org/10.1166/jnn.2020.17665.

Ishikawa, M., Yoshida, K., Okamura, H., Ochiai, K., Takamura, H., Fujiwara, N., Ozaki, K., 2013. Oral *Porphyromonas gingivalis* translocates to the liver and regulates hepatic glycogen synthesis through the Akt/GSK-3β signaling pathway. Biochim. Biophys. Acta Mol. Basis Dis. 1832, 2035–2043. https://doi.org/10.1016/j.bbadis.2013.07.012.

Islam, M.T., 2017. Oxidative stress and mitochondrial dysfunction-linked neurodegenerative disorders. Neurol. Res. 39, 73–82. https://doi.org/10.1080/01616412.2016.1251711.

Jacobsen, J., Nielsen, E.B., Brøndum-Nielsen, K., Christensen, M.E., Olin, H.B., Tommerup, N., Rassing, M.R., 1999. Filter-grown TR146 cells as an *in vitro* model of human buccal epithelial permeability. Eur. J. Oral Sci. 107, 138–146. https://doi.org/10.1046/j.0909-8836.1999.eos107210.x.

Jeong, H.R., Kim, K.J., Lee, S.G., Cho, H.S., Cho, Y.S., Kim, D.O., 2020. Phenolic profiles of hardy kiwifruits and their neuroprotective effects on pc-12 and sh-sy5y cells against oxidative stress. J. Microbiol. Biotechnol. 30, 912–919. https://doi.org/10.4014/jmb.2001.01047.

Kawai, M., Hirano, T., Higa, S., Arimitsu, J., Maruta, M., Kuwahara, Y., Ohkawara, T., Hagihara, K., Yamadori, T., Shima, Y., Ogata, A., Kawase, I., Tanaka, T., 2007. Flavonoids and related compounds as anti-allergic substances. Allergol. Int. 56, 113–123. https://doi.org/10.2332/allergolint.R-06-135.

Kebschull, M., Demmer, R.T., Papapanou, P.N., 2010. Gum bug, leave my heart alone!" - Epidemiologic and mechanistic evidence linking periodontal infections and atherosclerosis. J. Dent. Res. 89, 879–902. https://doi.org/10.1177/0022034510375281.

Kim, D., Kim, S.H., Park, E.J., Kang, C.Y., Cho, S.H., Kim, S., 2009a. Anti-allergic effects of PG102, a water-soluble extract prepared from *Actinidia arguta*, in a murine ovalbumin-induced asthma model. Clin. Exp. Allergy 39, 280–289. https://doi.org/10.1111/j.1365-2222.2008.03124.x.

Kim, D., Kim, S.H., Park, E.J., Kim, J., Cho, S.H., Kagawa, J., Arai, N., Jun, K., Kiyono, H., Kim, S., 2009b. Suppression of allergic diarrhea in murine ovalbumin-induced allergic diarrhea model by PG102, a water-soluble extract prepared from *Actinidia arguta*. Int. Arch. Allergy Immunol. 150, 164–171. https://doi.org/10.1159/000218119.

Kopustinskiene, D.M., Jakstas, V., Savickas, A., Bernatoniene, J., 2020. Flavonoids as anticancer agents. Nutr 12. https://doi.org/10.3390/nu12020457.

Kumar, K., Srivastav, S., Sharanagat, V.S., 2021. Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review. Ultrason. Sonochem. 70, 105325 https://doi.org/10.1016/j.ultsonch.2020.105325.

Kumar, N., Goel, N., 2019. Phenolic acids: Natural versatile molecules with promising therapeutic applications. Biotechnol. Reports 24, e00370. https://doi.org/10.1016/j.btre.2019.e00370.

Latocha, P., 2017. The nutritional and health benefits of kiwiberry (*Actinidia arguta*) – A review. Plant Foods Hum. Nutr. 72, 325–334. https://doi.org/10.1007/s11130-017-0637-y.

Latocha, P., Łata, B., Stasiak, A., 2015. Phenolics, ascorbate and the antioxidant potential of kiwiberry vs. common kiwifruit: The effect of cultivar and tissue type. J. Funct. Foods 19, 155–163. https://doi.org/10.1016/j.jff.2015.09.024.

Lin, G.C., Leitgeb, T., Vladetic, A., Friedl, H.P., Rhodes, N., Rossi, A., Roblegg, E., Neuhaus, W., 2020. Optimization of an oral mucosa *in vitro* model based on cell line TR146. Tissue Barriers 8, 1748459. doi:10.1080/21688370.2020.1748459.

Maritim, A.C., Sanders, R.A., Watkins Iii, J.B., 2003. Diabetes, oxidative stress, and antioxidants: A review. J. Biochem. Mol. Toxic. 17, 24–38. https://doi.org/10.1002/jbt.10058.

Nishiyama, I., Fukuda, T., Oota, T., 2005. Genotypic differences in chlorophyll, lutein, and beta-carotene contents in the fruits of *Actinidia* species. J. Agr. Food Chem. 53, 6403–6407. https://doi.org/10.1021/jf050785y.

Nishiyama, I., Fukuda, T., Shimohashi, A., Oota, T., 2008. Sugar and organic acid composition in the fruit juice of different *Actinidia* varieties. Food Sci. Technol. Res. 14, 67–73. https://doi.org/10.3136/fstr.14.67.

Panja, P., 2018. Green extraction methods of food polyphenols from vegetable materials. Curr. Opin. Food Sci. 23, 173–182. https://doi.org/10.1016/j.cofs.2017.11.012.

Peng, S.Y., Hsiao, C.C., Lan, T.H., Yen, C.Y., Farooqi, A.A., Cheng, C.M., Tang, J.Y., Yu, T. J., Yeh, Y.C., Chuang, Y.T., Chiu, C.C., Chang, H.W., 2020. Pomegranate extract inhibits migration and invasion of oral cancer cells by downregulating matrix metalloproteinase-2/9 and epithelial-mesenchymal transition. Environment. Toxicol. 35, 673–682. https://doi.org/10.1002/tox.22903.

Perricone, C., Ceccarelli, F., Matteo, S., Di Carlo, G., Bogdanos, D.P., Lucchetti, R., Pilloni, A., Valesini, G., Polimeni, A., Conti, F., 2019. *Porphyromonas gingivalis* and rheumatoid arthritis. Curr. Opin. Rheumatol. 31 https://doi.org/10.1097/BOR.0000000000000638.

Pinto, D., Sut, S., Dall'Acqua, S., Delerue-Matos, C., Rodrigues, F., 2021. *Actinidia arguta* pulp: Phytochemical composition, radical scavenging activity, and *invitro* cells effects. Chem. Biodivers. 18, e2000925 https://doi.org/10.1002/cbdv.202000925.

Pliszka, B., Huszcza-Ciołkowska, G., Wierzbicka, E., 2016. Effects of solvents and extraction methods on the content and antiradical activity of polyphenols from fruits *Actinidia arguta, Crataegus monogyna, Gaultheria procumbens* and *Schisandra chinensis*. Acta Sci. Pol. Technol. Aliment. 15, 57–63. https://doi.org/10.17306/j.Afs.2016.1.6.

Rahmawati, D., Anggraini, W., Djamil, M., 2021. Cytotoxicity of red fruit ethyl acetate extract (*Pandanus conoideus lam.*) on squamous cell carcinoma cell line (HSC-3). Sci. Dent. J. 5, 42–46. https://doi.org/10.4103/sdj.Sdj_57_20.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3.

Sari, L.M., Subita, G.P., Auerkari, E.I., 2018. Areca nut extract demonstrated apoptosis-inducing mechanism by increased caspase-3 activities on oral squamous cell carcinoma. F1000Research 7, 723. https://doi.org/10.12688/f1000research.14856.5.

Shen, S., Chen, X., Zhuo, Q., Ma, Y., Wang, J., Wang, L., Gong, Z., Huo, J., 2022. Integrating untargeted metabolites and targeted analysis for discrimination of kiwifruits from different cultivars. J. Food Comp. Anal. 112, 104661 https://doi.org/10.1016/j.jfca.2022.104661.

Silva, A.M., Pinto, D., Fernandes, I., Gonçalves Albuquerque, T., Costa, H.S., Freitas, V., Rodrigues, F., Oliveira, M.B.P.P., 2019. Infusions and decoctions of dehydrated fruits of *Actinidia arguta* and *Actinidia deliciosa*: Bioactivity, radical scavenging activity and effects on cells viability. Food Chem 289, 625–634. https://doi.org/10.1016/j.foodchem.2019.03.105.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vit. 16, 144. http://www.ajevonline.org/content/16/3/144.abstract.

Sosa, V., Moliné, T., Somoza, R., Paciucci, R., Kondoh, H., Lleonart, M.E., 2013. Oxidative stress and cancer: An overview. Ageing Res. Rev. 12, 376–390. https://doi.org/10.1016/j.arr.2012.10.004.

Suzuki, R., Matsushima, Y., Okudaira, N., Sakagami, H., Shirataki, Y., 2016. Cytotoxic components against human oral squamous cell carcinoma isolated from *Andrographis paniculata*. Anticancer Res 36, 5931–5935. https://doi.org/10.21873/anticanres.11180.

Van Tyne, D., Martin, M.J., Gilmore, M.S., 2013. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. Toxins 5, 895–911. https://doi.org/10.3390/toxins5050895.

Wang, Y., Zhao, C.-l., Li, J.-y., Liang, Y.-j., Yang, R.-q., Liu, J.-y., Ma, Z., Wu, L., 2018. Evaluation of biochemical components and antioxidant capacity of different kiwifruit (*Actinidia* spp.) genotypes grown in China. Biotechnol. Biotechnol. Equip. 32, 558–565. https://doi.org/10.1080/13102818.2018.1443400.

Wojdyło, A., Nowicka, P., Oszmiański, J., Golis, T., 2017. Phytochemical compounds and biological effects of *Actinidia* fruits. J. Funct. Foods 30, 194–202. https://doi.org/10.1016/j.jff.2017.01.018.

Xiangxue, A., Sang Gil, L., Hee, K., Ho Jin, H., Youn-Sup, C., Dae-Ok, K., 2016. Antioxidant and anti-inflammatory effects of various cultivars of kiwi berry (*Actinidia arguta*) on lipopolysaccharide-stimulated RAW 264.7 cells. J. Microbiol. Biotechnol. 26, 1367–1374. https://doi.org/10.4014/jmb.1603.03009.

Yu, X., Liu, C., Liu, Y., Tan, C., 2015. *Actinidia arguta* polysaccharide induces apoptosis in Hep G2 cells. Adv. J. Food Sci. Technol. 7, 857–863. https://doi.org/10.19026/ajfst.7.2522.

Zhang, J., Gao, N., Shu, C., Cheng, S., Sun, X., Liu, C., Xin, G., Li, B., Tian, J., 2021. Phenolics profile and antioxidant activity analysis of kiwi berry (*Actinidia arguta*) flesh and peel extracts from four regions in China. Frontiers Plant Sci 12. https://doi.org/10.3389/fpls.2021.689038.

Zhang, J., Gao, N., Jiang, H., Li, H., Cui, X., Tang, S., Jin, C., Tian, J., Li, B., 2022. Comparative study on alleviating effect of kiwi berry (*Actinidia arguta*) polysaccharide and polyphenol extracts on constipated mice. Food Res. Int. 162, 112037 https://doi.org/10.1016/j.foodres.2022.112037.

Zhou, Y., Luo, G.H., 2019. *Porphyromonas gingivalis* and digestive system cancers. World J. Clin. Cases 7, 819–829. https://doi.org/10.12998/wjcc.v7.i7.819.

Zuo, L.-L., Wang, Z.-Y., Fan, Z.-L., Tian, S.-Q., Liu, J.-R., 2012. Evaluation of antioxidant and antiproliferative properties of three Actinidia (*Actinidia kolomikta, Actinidia arguta, Actinidia chinensis*) extracts *in vitro*. Int. J. Mol. Sci. 13, 5506–5518. https://doi.org/10.3390/ijms1305550.