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ORIGINAL ARTICLE

Enhanced ultrasonically assisted extraction of bitter melon (*Momordica charantia*) leaf phenolic compounds using choline chloride-acetic acid–based natural deep eutectic solvent: an optimization approach and in vitro digestion

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Abstract
Bitter melon (*Momordica charantia*) is a rich source of phytochemicals including phenolic compounds with diverse health-promoting benefits and potential food industry application due to their antioxidant potential. Bitter melon leaves have been limitedly investigated in comparison to bitter melon fruits. The current work explores the use of green extraction methodology to optimize enhanced extraction of phenolic compounds from bitter melon leaves using ultrasonically assisted extraction and choline chloride-acetic acid (CHAC)–based natural deep eutectic solvent. Extraction using CHAC significantly improved the extraction of total phenolic compounds, total flavonoids, and individual phenolic compounds (including gallic acid, chlorogenic acid, vanillic acid, epicatechin, and quercetin-3-glucoside) in comparison to water, ethanol, and methanol. The effect of molar ratio, water content, temperature, and time on the extraction efficiency of bitter melon leaf phenolic compounds by CHAC was explored and optimized with surface response methodology (central composite design). The optimum condition for the extraction of individual phenolic compounds is a molar ratio of 1:4.35 CHAC with 20.68% water content at 75 °C for 21.23 min. Evaluation of the bioaccessibility of individual phenolic compounds concluded that the most bioaccessible compound was vanillic acid (105.00 ± 2.52%) followed by salicylic acid, chlorogenic acid, syringic acid, gallic acid, epicatechin, and quercetin-3-glucoside.

Keywords Natural deep eutectic solvent · Bitter melon · Phenolic compounds · Surface response methodology · Extraction · Optimization

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1 Introduction

Momordica charantia or bitter melon is a worldwide plant, used as a vegetable and in folk medicine. It belongs to the Cucurbitaceae family and grows mainly in tropical areas of Africa Asia, and the Amazon [1, 2]. It produces oblong fruits that are yellow to orange in colour when ripe. Mature fruits are traditionally applied for healing wounds and ulcers. Also, their green jagged-edge leaves have been applied for the treatment of many ailments believing in the beneficial health effects [1, 2]. Furthermore, flowers, roots, stems and seeds are not less beneficial parts [3, 4].

All the previous plant parts contain a considerable amount of biologically active compounds like flavonoids, saponins, alkaloids, tannins, and glycosides [1, 5, 6]. Those chemical compounds permit the application of an array of positive activities such as antioxidant, anti-cancer, anti-inflammatory,

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anti-microbial and anti-viral activities [2, 4]. For instance, the fruit contains momordicins, momorcharins, cucurbitins, elaeostearic acids, etc. [1]. Similarly, leaves besides momordicins [7] contain an important amount of phenolics such as gallic acid, followed by caffeic acid and catechin [4]. It has been reported that bitter melon leaves have higher phenolic content in comparison to bitter melon fruit [8]. Also, *Momordica* leaves are a good source of vitamins (A, E, B and C), minerals (K^+, Ca^{2+}, Na^{2+} and Zn^{2+}) and carbohydrates, making them nutritionally valuable as well [6].

The process of extracting the different phytochemicals from plant material has been applied since ancient times to take advantage of their beneficial and nutritional activities. Extraction studies of *Momordica* parts, using conventional to highly sophisticated techniques, have demonstrated interesting results to better understand the methods of recovery of bioactive compounds as well as their mode of action [2–4, 9].

Conventional organic solvents such as ethanol, methanol, acetone, ethyl acetate and chloroform have been widely used for the extraction of bioactive compounds including phenolic compounds. These solvents are associated with toxicity, high prices, hazards and unsustainability. Natural deep eutectic solvents (NADESs) are considered an alternative green solvent that has been used efficiently to extract a wide range of phenolic compounds from different matrices with enhanced extraction yields [10–12]. NADES are easy to prepare, have good biodegradability and sustainability and have low costs that make them fully meet green chemistry principles [13, 14]. NADESs are made up of a hydrogen bond donor (HBD) and a hydrogen bond acceptor (HBA) that together form a supramolecular structure with hydrogen bonds [11]. Their enhanced extraction potential of phenolic compounds is associated with their ability to form hydrogen bonds with phenolic compounds and increase their solubility [15, 16].

The ultrasound-assisted extraction (UAE) utilizes the acoustic cavitation to generate bubbles during the period of negative pressure [17, 18]. The bubbles are compressed leading to their collapse, further disrupting the solid cell walls and allowing the release of bioactive compounds [19, 20]. The UAE offers many advantages such as improved efficiency, reduced extraction time, low solvent consumption and high level of automation compared to conventional extraction method [19, 21, 22].

The acid-based NADESs were revealed to be efficient for the recovery of phenolic compounds due to their strong hydrogen bonding with phenolic compounds, their solubilizing capacity of phenolic compounds and their low viscosity [23, 24]. To the best of our knowledge, no studies have applied the use of NADES in extracting phenolic compounds from bitter melon leaves and have optimized the extraction conditions of those phenolic compounds using choline chloride-acetic acid NADES. Therefore, in this study, ultrasonically assisted extraction was used with choline chloride-acetic acid (CHAC)–based NADES to enhance the extraction of phenolic compounds from bitter melon leaves. The extraction efficiency of CHAC was compared to other conventional extracting solvents (methanol, ethanol and water). Furthermore, the optimum conditions for extraction of individual phenolic compounds (gallic acid, chlorogenic acid, vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside and salicylic acid) (Fig. 1) were determined by applying surface response methodology (central composite design). In addition, the in vitro bioaccessibility of these compounds was determined.

2 Material and methods

2.1 Plant material

Bitter melon (*Momordica charantia*) leaves were collected in August 2021 from Abomey-Calavi, Benin. The leaves were shade-dried for five days, sorted and packed in brown bottles and screw caps.

2.2 Chemical and reagents

Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol (HPLC grade), ethanol (HPLC grade), acetonitrile (HPLC grade), sodium carbonate, sodium acetate, sodium nitrite, sodium hydroxide, hydrochloric acid (37%) and choline chloride (≥ 98%) and standards were purchased from Sigma-Aldrich Chemical Co. (St Louis, MO). Gallic acid, glacial acetic acid (≥ 99.5%) and iron sulphate were purchased from Carlo Erba. Aluminium chloride and iron chloride were acquired from Merck while glycerol (≥ 99.5%) was purchased from Tekkim.

2.3 Preparation of NADES

The NADES was prepared according to [19]. The HBA (choline chloride) and HBD (acetic acid) were combined at a 1:2 molar ratio, followed by the addition of 20% of distilled water. Afterwards, the mixture was heated for 2 h and 30 min at 80 °C to obtain homogenized liquid encode as CHAC.

2.4 Extraction of phenolic compounds

The extraction was carried out using an ultrasonic water bath. Distilled water, methanol and ethanol (conventional solvents) and CHAC (NADES with 20% water) were used as solvents. An amount of 0.3 g was mixed with 10 mL of solvents, and the mixture was ultrasonicated at 25 °C for 20 min. The samples were then filtered through Whatman filter paper No. 1 thrice.

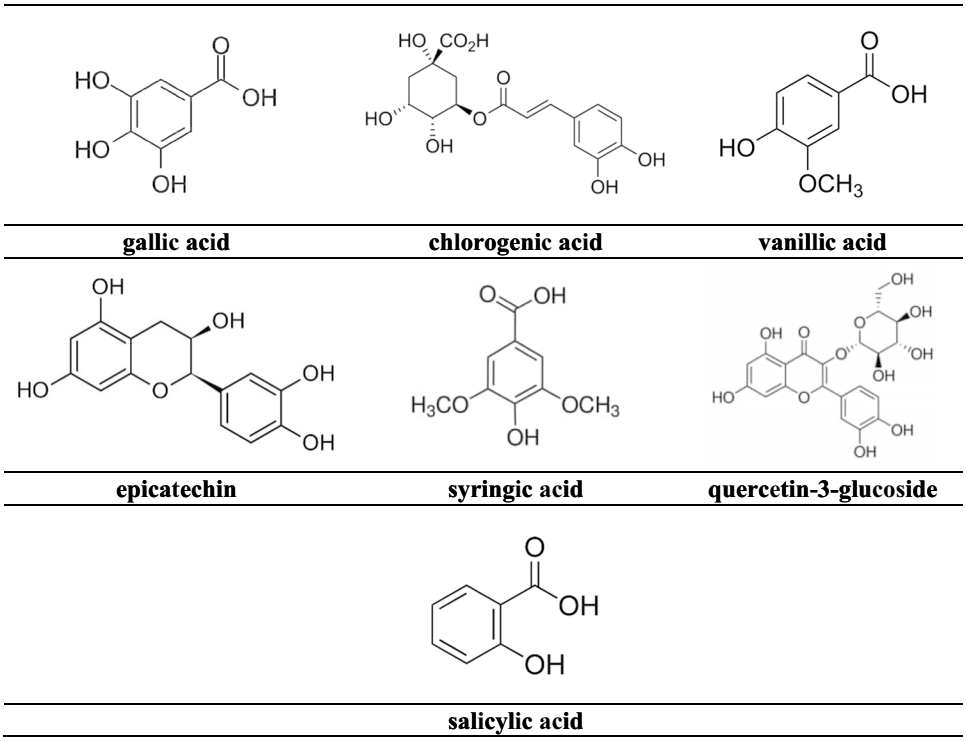


Fig. 1 Structure of gallic acid, chlorogenic acid, vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside, and salicylic acid

2.5 Total phenolic content

Total phenolic content (TPC) was evaluated by the Folin-Ciocalteu method adopted from Nguyen et al. [25] with some modifications. Briefly, 150 µL of samples was mixed with 750 µL of 10% Folin-Ciocalteu reagent (5 min) and 600 µL of 7.5% Na_2CO_3. The mixture was kept in dark for 2 h, and the absorbance was read at 760 nm. TPC was expressed as milligrams of gallic acid equivalent per gram (mg GAE/g).

2.6 Total flavonoid content

Total flavonoid content (TFC) was determined according to the method of Zannou and Koca [26]. Briefly, 1 mL of the appropriately diluted sample was mixed with 300 µL of 5% $NaNO_2$ and 500 µL of 5% $AlCl_3$ and 500 µL of 1 M NaOH respectively. Afterwards, the mixture was placed in the dark for 10 min and the absorbance was read at 510 nm. The results were given as milligrams of epicatechin equivalents per gram (mg ECE/g).

2.7 Determination of antioxidant activity

2.7.1 Diphenyl-1-picrylhydrazyl radical scavenging activity assay

DPPH assay was conducted following the method of Zannou and Koca [26]. The absorbance was read against a control. The values of DPPH radical scavenging were determined with a calibration curve as millimoles of Trolox equivalent per gram (mmol TE/g).

2.7.2 Ferric reducing antioxidant power assay

Ferric reducing antioxidant power (FRAP) assay was conducted following the method of Zannou and Koca [26]. The value of FRAP was obtained from a standard curve of $FeSO_4$. The results were given as millimoles of $FeSO_4$ equivalents per gram (mmol ISE/g).

2.8 Determination of individual phenolic compounds

The individual phenolic compounds were identified using the previous method of [27] with modifications. The phenolic compounds were determined using a high-pressure liquid chromatography (HPLC) system (Agilent 1260; Agilent Technologies) with a diode array detector (DAD) at 520-nm wavelength. The anthocyanins were separated in an Inertsil ODS-4 column (3 µm, 4,6 × 50 mm; GL Sciences Kat No: 5020–0404) at a 1 mL min^{-1} flow rate. The mobile phases were: (A) 94% 2 mM sodium acetate and 6% acetic acid (v/v) and (B) acetonitrile. The following elution gradient was used, according to solvent B: 0–20 min, 14–23%; 20–40 min, 23–35%; 40–50 min, 40%; 50–60 min, 60%; 60–65 min, 95%. The column temperature was set at 30 °C. The individual phenolic compounds were identified

by comparing their retention times with their respective standard. The identified phenolic compounds were quantified using a mixture of external standards (gallic acid, chlorogenic acid, epicatechin, vanillic acid, syringic acid, quercetin-3-glucoside and salicylic acid) which were prepared at different concentrations.

2.9 Optimization with the response surface method

The optimization parameters were examined systematically using response surface methodology based on the three-level central composite design (Design-Expert software 13.0). The experimental design included four independent variables X_1 (CHAC, molar ratio), X_2 (water content, %), X_3 (temperature, °C) and X_4 (extraction time, min). The actual and coded values of the independent variables are shown in Table 1. The combination of parameters such as molar ratio of CHAC (1:0.5, 2, 3.5, 5 and 6.5), water content (10, 20, 30, 40 and 50%), temperature (25, 40, 60, 75 and 90 °C) and extraction time (5, 15, 25, 35 and 45 min) were chosen as independent variables. From these variables, the response surface method (RSM) has generated 27 experimental points including three replicates at the central point. Nine responses (Y) were considered, namely gallic acid, chlorogenic acid, epicatechin, vanillic acid, syringic acid, quercetin-3-glucoside and salicylic acid. The experimental points together with responses are shown in Table 2. The analyses were carried out in triplicate, and the results were shown as means ± standard deviation. The experimental data were fitted to the following quadratic polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_{ii} + \sum_{i=1}^{k-1} \sum_{i=i+1}^{k} \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

where Y is the response; X is the independent variable; β_0 is the model intercept coefficient; β_i, β_{ii}, and β_{ij} are interaction coefficients; k is the number of independent factors; and ε is the experimental error. The relationship between independent variables and responses was examined using analysis of variance (ANOVA) test in the Design-Expert program.

Table 1 Actual and coded values of independent variables

	Actual values			
	X_1	X_2	X_3	X_4
−1.41	0.5	10	25	5
−1	2	20	40	15
0	3.5	30	60	25
+1	5	40	75	35
+1.41	6.5	50	90	45

X_1, molar ratio; X_2, water content, %; X_3, temperature, °C; X_4, extraction time, min

2.10 In vitro *bioaccessibility*

The in vitro bioaccessibility of phenolic compounds was determined to be the fraction of phenolic compounds that were solubilized within the mixed micelles and which became accessible for intestinal absorption [28]. Following in vitro digestion, an aliquot of raw digesta was collected after the simulated small intestine digestion and centrifuged at 5000 × g for 15 min at 4 °C. A supernatant (micelle fraction) was collected from the centrifuged digesta in which the anthocyanins were solubilized. A portion (3 mL) of the micelle fraction was vortexed after adding 3 mL of methanol and centrifuged at 5000 × g for 15 min at 25 °C. The supernatant was then carefully collected and used for the determination of phenolic compounds using HPLC–DAD. The bioaccessibility and stability of phenolic compounds were then determined using the following equations:

$$\text{Bioaccessibility}(\%) = (C_{\text{Micelle}}/C_{\text{Initial}}) \times 100 \quad (2)$$

where C_{Initial} and C_{Micelle} are the concentration of the individual phenolic compounds initially and in the micelle phase at the end of the in vitro digestion, respectively.

2.11 Statistical analyses

All results were expressed as the mean of three replicates ± standard deviation. Statistical analyses were performed using a one-way analysis of variance ANOVA, and the significance of the difference between means was evaluated by Turkey's test. Statistical significance was determined at $p < 0.05$. Design-Expert software (version 13.0, Stat-Ease Inc., Minneapolis, USA) was used for the RSM and experimental data analysis. ANOVA was used to determine the statistical relationship between factors. The adequacy of the models was determined by R^2, adjusted R^2, predicted R^2, coefficient of variation (CV), adequate precision, p value and the value of Fisher's test (F value). The significance of the models and regression coefficients were measured at $p < 0.05$. The behaviours of variables and responses were checked by the perturbation graphics. The optimum conditions were determined by applying the desirability function.

3 Results and discussion

3.1 Efficiency of CHAC in comparison to conventional solvents

The phytochemical characteristics such as pH, viscosity, FTIR and electric conductivity of the choline chloride-acetic acid–based NADES with 20% water (CHAC) used in

Table 2 ANOVA results of TPC, TFC, DPPH, FRAP and targeted individual phenolic compounds

	TPC			TFC			DPPH			FRAP			Gallic acid			Chlorogenic acid		
	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value
Model	945.96	8.12	< 0.001	15.98	20.71	< 0.001	18,211.58	11.49	< 0.001	11,446.10	11.97	< 0.001	123,603.51	15.54	< 0.001	111.63	19.78	< 0.001
X_1	179.08	21.53	0.001	1.12	20.36	0.001	55.43	0.49	0.498	20.16	0.30	0.597	1092.35	1.92	0.191	21.24	52.71	< 0.001
X_2	4.03	0.48	0.500	5.07	92.07	< 0.001	106.18	0.94	0.352	10.73	0.16	0.699	8228.64	14.48	0.003	0.21	0.52	0.486
X_3	502.12	60.36	< 0.001	0.36	6.56	0.025	4286.83	37.85	< 0.001	180.48	2.64	0.130	81,702.25	143.80	< 0.001	1.94	4.82	0.048
X_4	2.52	0.30	0.592	3.36	60.99	< 0.001	3651.34	32.24	< 0.001	3.06	0.04	0.836	9102.82	16.02	0.002	1.56	3.86	0.073
$X_1 X_2$	3.09	0.37	0.554	0.02	0.31	0.586	636.17	5.62	0.035	1753.66	25.67	< 0.001	26.25	0.05	0.833	7.29	18.10	0.001
$X_1 X_3$	37.58	4.52	0.055	0.99	17.96	0.001	970.47	8.57	0.013	1452.36	21.26	0.001	41.37	0.07	0.792	0.09	0.24	0.636
$X_1 X_4$	30.57	3.68	0.079	0.21	3.88	0.073	776.28	6.85	0.022	659.48	9.65	0.009	317.60	0.56	0.469	16.09	39.93	< 0.001
$X_2 X_3$	11.47	1.38	0.263	0.01	0.09	0.765	373.85	3.30	0.094	1.85	0.03	0.872	1009.48	1.78	0.207	2.12	5.27	0.041
$X_2 X_4$	13.02	1.56	0.235	3.11	56.50	< 0.001	2498.11	22.06	0.001	56.25	0.82	0.382	1892.14	3.33	0.093	3.63	9.00	0.011
$X_3 X_4$	0.79	0.09	0.763	0.19	3.45	0.088	990.58	8.75	0.012	633.09	9.27	0.010	4550.72	8.01	0.015	33.52	83.17	< 0.001
$X_1 X_1$	0.15	0.02	0.894	0.27	4.87	0.048	821.84	7.26	0.020	160.86	2.35	0.151	8687.79	15.29	0.002	0.75	1.87	0.196
$X_2 X_2$	33.33	4.01	0.068	0.53	9.69	0.009	22.84	0.20	0.661	4397.36	64.36	< 0.001	999.75	1.76	0.209	4.08	10.13	0.008
$X_3 X_3$	4.05	0.49	0.498	0.00	0.02	0.893	1509.17	13.33	0.003	565.59	8.28	0.014	1771.36	3.12	0.103	9.26	22.98	< 0.001
$X_4 X_4$	59.84	7.19	0.020	0.23	4.08	0.066	2928.08	25.85	< 0.001	116.15	1.70	0.217	68.43	0.12	0.735	5.16	12.80	0.004
Residual	99.82			0.66			1359.05			819.85			6817.89			4.84		
Lack of fit	71.51	0.51	0.811	0.65	17.72	0.055	571.91	0.15	0.987	730.29	1.63	0.439	5273.79	0.68	0.723	4.14	1.19	0.539
Pure error	28.32			0.01			787.14			89.56			1544.10			0.69		
Cor total	1045.78			16.64			19,570.62			12,265.96			130,421.40			116.46		
R^2	0.905			0.960			0.931			0.933			0.948			0.958		
Adj R^2	0.793			0.914			0.850			0.855			0.887			0.910		
Pred R^2	0.558			0.767			0.737			0.641			0.744			0.777		
Adeq precision	9.23			15.73			13.48			13.77			13.03			17.81		
C.V. %	12.40			25.99			10.86			5.79			8.94			1.58		

	Vanillic acid			Epicatechin			Syringic acid			Quercetin-3-glucoside			Salicylic acid		
	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value	SS	*F* value	*p* value
Model	1483.73	26.08	< 0.001	15,707.77	10.82	< 0.001	291.28	15.63	< 0.001	4200.73	13.21	< 0.001	51,447.20	16.01	< 0.001
X_1	6.36	1.56	0.235	4789.13	46.18	< 0.001	53.57	40.24	< 0.001	418.11	18.41	0.001	15,631.96	68.10	< 0.001
X_2	126.54	31.14	< 0.001	308.79	2.98	0.110	0.81	0.61	0.451	685.27	30.18	< 0.001	694.25	3.02	0.108
X_3	1.59	0.39	0.543	172.48	1.66	0.221	168.73	126.73	< 0.001	597.58	26.32	< 0.001	4082.48	17.79	0.001
X_4	0.00	0.00	0.997	1330.60	12.83	0.004	4.32	3.25	0.097	0.33	0.01	0.906	4058.24	17.68	0.001
$X_1 X_2$	577.98	142.21	< 0.001	159.79	1.54	0.238	0.63	0.47	0.506	0.01	0.00	0.983	1153.71	5.03	0.045
$X_1 X_3$	20.81	5.12	0.043	32.05	0.31	0.588	0.52	0.39	0.543	90.89	4.00	0.069	884.00	3.85	0.073
$X_1 X_4$	4.14	1.02	0.333	236.70	2.28	0.157	0.96	0.72	0.414	32.87	1.45	0.252	5545.22	24.16	< 0.001
$X_2 X_3$	0.07	0.02	0.897	1699.93	16.39	0.002	5.77	4.33	0.060	24.38	1.07	0.321	2655.40	11.57	0.005
$X_2 X_4$	176.95	43.54	< 0.001	94.82	0.91	0.358	15.87	11.92	0.005	543.65	23.94	< 0.001	475.95	2.07	0.175
$X_3 X_4$	27.81	6.84	0.023	127.87	1.23	0.289	5.99	4.50	0.055	10.70	0.47	0.505	65.14	0.28	0.604

Table 2 (continued)

	Vanillic acid			Epicatechin			Syringic acid			Quercetin-3-glucoside			Salicylic acid		
X_1X_1	31.46	7.74	0.017	3516.53	33.91	< 0.001	11.10	8.34	0.014	224.18	9.87	0.009	6141.58	26.76	< 0.001
X_2X_2	454.22	111.76	< 0.001	6.57	0.06	0.806	11.36	8.53	0.013	395.36	17.41	0.001	711.88	3.10	0.104
X_3X_3	8.24	2.03	0.180	3832.50	36.96	< 0.001	1.28	0.96	0.346	518.23	22.82	< 0.001	2011.15	8.76	0.012
X_4X_4	183.35	45.11	< 0.001	215.82	2.08	0.175	0.01	0.00	0.952	2.79	0.12	0.732	3063.55	13.35	0.003
Residual	48.77			1244.39			15.98			272.50			2754.35		
Lack of fit	47.06	5.50	0.164	964.92	0.69	0.720	13.82	1.28	0.515	223.15	0.90	0.632	2578.37	2.93	0.281
Pure error	1.71			279.46			2.15			49.35			175.98		
Cor total	1532.50			16,952.16			307.26			4473.23			54,201.55		
R^2	0.968			0.927			0.948			0.939			0.949		
Adj R^2	0.931			0.841			0.887			0.868			0.890		
Pred R^2	0.820			0.642			0.738			0.683			0.715		
Adeq precision	17.75			11.41			15.12			16.39			16.89		
C.V. %	2.99			3.94			5.28			6.18			4.55		

the present study have been reported in our previous study [29]. Moreover, CHAC has been revealed to be efficient and eco-friendly for the extraction of phenolic compounds from various plant materials [23, 30].

3.1.1 Total phenolic compounds and antioxidant potential

The phenolic content and antioxidant capacity of bitter melon extracts obtained using different solvents (CHAC, water, ethanol and methanol) were investigated and compared (Table 3). Based on the findings, the total phenolic of CHAC extract was significantly the highest ($p < 0.05$) and most efficient yielding 82.06 ± 1.97 mg GAE/g, followed by water (11.80 ± 0.01 mg GAE/g) and organic solvents (< 7 mg GAE/g). On the other hand, the total flavonoid content extracted by water from bitter melon (1.84 ± 0.20 mg ECE/g) was significantly higher than CHAC extract (1.26 ± 0.01 mg ECE/g) ($p < 0.05$), suggesting that the flavonoids extracted from bitter leaves could be more hydrophilic. Total flavonoids seem to contribute very limitedly to the total phenolic content, suggesting that the majority of the phenolic compounds present in the bitter melon extract are non-flavonoids. It has previously been reported that gallic acid, chlorogenic acid, gentisic acid, vanillin acid, catechin, epicatechin, protocatechuic acid, t-cinnamic acid, p-coumaric acid, and o-coumaric acid are the most common phenolic compounds present in bitter melon fruit [31]. Out of these compounds, only catechin and epicatechin are flavonoids. Phenolic compounds have a wide range of health-promoting benefits and are mainly investigated for their antioxidant potential. The antioxidant capacity of the bitter melon extracts was investigated and compared by two in vitro assays (DPPH and FRAP). The CHAC extract showed significantly higher ($p < 0.05$) antioxidant activity (DPPH assay, 80.67 ± 3.94 mmol TE/g, and FRAP assay, 228.27 ± 5.14 mmol ISE/g) in comparison to other extracts. The other extracts showed a DPPH radical scavenging activity of 5–12 mmol TE/g and FRAP values of 130–175 mmol ISE/g. The improved antioxidant activity of CHAC extract can be associated with the higher phenolic content of that extract. It has been reported that acidic NADES such as CHAC are capable of extracting a high amount of anthocyanins that have strong radical scavenging activity [29].

The current study is the first study to apply a natural deep eutectic solvent system for the extraction of bitter melon leaf phenolic compounds and investigate their antioxidant capacity. Yet, the efficiency of CHAC solvent used in this study has shown greater efficiency in extracting phenolic compounds in comparison to other extracting solvents that have been reported earlier. For example, bitter melon leaves have been previously extracted by

Table 3 Phenolic content and antioxidant capacity of CHAC, water, ethanol and methanol extracts of bitter melon

Solvents	Phenolic content		Antioxidant capacity	
	Total phenolic content (mg GAE/g)	Total flavonoid content (mg ECE/g)	DPPH (mmol TE/g)	FRAP (mmol ISE/g)
CHAC	82.06 ± 1.97^{a}	1.26 ± 0.01^{b}	80.67 ± 3.94^{a}	228.27 ± 5.14^{a}
Water	11.80 ± 0.01^{b}	1.84 ± 0.20^{a}	11.80 ± 0.01^{b}	132.91 ± 1.02^{d}
Ethanol	5.93 ± 0.32^{c}	0.72 ± 0.01^{c}	5.75 ± 0.65^{c}	173.68 ± 1.03^{b}
Methanol	6.21 ± 0.01^{c}	0.74 ± 0.03^{c}	6.21 ± 0.01^{c}	144.56 ± 1.03^{c}

Lowercase letters a–d in the same column mean a significant difference at $p < 0.05$

solid–liquid extraction using a series of solvents, starting with an acidic methanol solvent followed by aqueous methanol and ethyl acetate [32]. The total phenolic content extracted was 58.7 ± 1.8 mg GAE/g which is comparably less than the amount extracted by CHAC in this study. Other studies have reported lower total phenolic content in comparison to the amount extracted by CHAC. Methanolic extraction of two different varieties of bitter melon leaves by maceration has previously yielded 25.86 ± 0.36 and 25.94 ± 0.35 mg GAE/g with DPPH half-maximal inhibitory concentrations (IC_{50}) of 28.00 ± 0.83 and 20.39 ± 1.12 mg/mL, respectively [33]. The total phenolic content of ethanolic maceration of bitter melon leaves has previously been reported as 25.297 ± 0.146 [34] and 45.55 ± 0.34 mg GAE/g [8]. The total flavonoid content of bitter melon leaves in the current study was less than that reported in other studies regardless of the nature of the solvent. For example, the total flavonoid content of ethanolic maceration of bitter melon leaves yielded 11.945 mg RE (rutin equivalents)/g [34] and 47.25 ± 1.48 mg CA (catechins)/g [8]. These differences could be associated with the variety and growth conditions of those plants. Moreover, optimized methanolic maceration conditions for extracting phenolic compounds from bitter melon leaves have reported a lower a total phenolic content (20.66 mg GAE/g) compared to the phenolic content extracted by CHAC, with a DPPH scavenging activity of 30.22 mg TE/g and FRAP value of 45.48 mg TE/g [9].

The superior extracting efficiency of CHAC has also been observed in other studies using different plant matrices to extract phenolic compounds. CHAC showed higher extraction efficiency compared to other NADESs (choline chloride-based malonic, malic and citric acid NADESs) in extracting phenolic compounds from olive leaves [30]. The authors reported that the enhanced extraction potential of CHAC compared to others is due to its reduced density and viscosity. Monocarboxylic acid (such as acetic acid)–based NADESs have shown higher extraction potential of phenolic compounds compared to di- and tri-carboxylic acid–based NADES when tested to extract *Juglans regia* L. phenolic compounds [35].

3.1.2 Individual phenolic compounds

The individual phenolic compounds present in different bitter melon extracts were quantified by chromatographic analysis and compared (Table 4). The highest amount of gallic acid, chlorogenic acid, vanillic acid, epicatechin and quercetin-3-glucoside was quantified in CHAC extract and was significantly greater than in other extracts ($p < 0.05$), while the highest amount of syringic acid was quantified in the water extract and was significantly greater than the other extracts ($p < 0.05$). The extraction efficiency of salicylic acid by CHAC and water was similar, and the difference was not significant ($p > 0.05$). The organic solvents showed an overall lower efficiency in

Table 4 Quantified individual phenolic compounds (mg/kg) of bitter melon extracts

Phenolic compounds	Retention time (min)	Solvents			
		CHAC	Water	Methanol	Ethanol
Gallic acid	4.72	311.25 ± 1.61^{a}	154.71 ± 0.72^{b}	42.62 ± 0.80^{c}	21.07 ± 0.91^{d}
Chlorogenic acid	17.08	40.52 ± 0.56^{a}	31.10 ± 1.25^{b}	28.2 ± 0.43^{c}	20.98 ± 0.04^{d}
Vanillic acid	19.41	81.49 ± 0.72^{a}	26.51 ± 0.58^{b}	25.69 ± 0.14^{b}	24.96 ± 0.70^{b}
Epicatechin	21.87	373.12 ± 7.07^{a}	117.90 ± 1.41^{b}	75.02 ± 0.63^{c}	60.44 ± 0.70^{d}
Syringic acid	24.08	21.73 ± 0.69^{b}	50.68 ± 0.60^{a}	10.77 ± 0.18^{c}	9.27 ± 0.25^{d}
Quercetin-3-glucoside	40.73	119.95 ± 0.35^{a}	15.59 ± 0.68^{c}	41.43 ± 0.73^{b}	16.10 ± 0.28^{c}
Salicylic acid	42.59	337.43 ± 3.28^{a}	361.43 ± 1.88^{a}	129.13 ± 1.45^{c}	64.98 ± 1.23^{b}

Lowercase letters a–d in the same row mean a significant difference at $p < 0.05$

extracting bitter melon phenolic compounds in comparison to the natural deep eutectic solvent and water.

To the best of our knowledge, the current study is the first study to report the amount of vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside and salicylic acid of bitter melon leaves and compare their yield while using different extracting solvents. Very few studies have previously reported the quantities of individual phenolic compounds in bitter melon leaves. The bitter melon leaf phenolic compounds obtained by solid–liquid extraction using a series of solvents, starting with an acidic methanol solvent followed by aqueous methanol and ethyl acetate, have been quantified [32]. The study reported that the extract contained gallic acid (0.03 mg/g), chlorogenic acid (1.36 mg/g), caffeic acid (0.05 mg/g), ferulic acid (0.05 mg/g), cinnamic acid (0.05 mg/g), myricetin (0.12 mg/g), quercetin (0.26 mg/g), luteolin (0.01 mg/g), apigenin (0.68 mg/g) and thymol (5.88 mg/g). Comparing these results with the current study, the gallic acid extracted by CHAC is largely greater than the previously reported content. However, the chlorogenic acid reported in the previous study is largely greater than the amount extracted in any of the solvents of the current study. In another study, aqueous extraction of bitter melon leaves yielded gallic acid (95.8 ± 0.31 mg/l), tannic acid (2.13 ± 0.67 mg/l), catechin (4.39 ± 0.80 mg/l), caffeic acid (7.77 ± 1.02 mg/l), *p*-coumaric acid (0.36 ± 0.32 mg/l) and benzoic acid (0.10 ± 0.42 mg/l) [4].

3.2 Optimizing the extraction process with RSM

The selection of optimum conditions for high extraction efficiency of total phenolic compounds (TPC, TFC), individual phenolic compounds and their related antioxidant activities (DPPH, FRAP) was realized by applying a central composite design. The independent variables chosen for this optimization are molar ratio (X_1), water content (X_2), temperature (X_3) and extraction time (X_4). Total and individual phenolic compounds content and the antioxidant activity of the obtained extracts are the responses of this optimization.

As shown in Table 2, all responses varied at all experimental points. TPC (13.05–36.81 mg/g), TFC (0.06–2.82 mg/g), DPPH (50.72–164.38 mmol TE/g) and FRAP (88.57–182.05 mmol ISE/g) demonstrated a large variation under the 27 generated points. Also, individual phenolic compounds such as gallic acid (172.5–407.02 mg/kg), chlorogenic acid (35.29–44.06 mg/kg), vanillic acid (51.86–81.00 mg/kg), epicatechin (195.99–297.32 mg/kg), syringic acid (15.51–29.8 mg/kg), quercetin-3-glucoside (44.43–109.35 mg/kg) and salicylic acid (185.2–389.62 mg/kg) had different contents at different conditions of extraction. The extraction efficiency of the studied parameters did not show a clear relation with the effects of independent variables. Except chlorogenic and vanillic acid at run 1 (1:5 molar ratio, 40% of water content, 40 °C and 35 min), all the responses had various runs for best extraction efficiency. This dispersion of efficient points of extraction could translate the variation of required conditions for best recovery of the concerned compounds.

ANOVA results for the obtained experimental points are shown in Table 2. All the responses exhibited high suitability with the quadratic model. The model had high significance ($p < 0.0001$) for most responses. Lack of fit was insignificant for all the responses ($p > 0.0545$). R^2 and adjusted-R^2 were sufficiently high, > 0.905 and > 0.793, respectively. All these parameters confirm the adequacy of the quadratic model for navigation in the design space.

The model terms of all responses are shown in Table 2. Generally, all of the individual phenolic compounds including TPC, TFC and DPPH showed at least one significant linear term, except FRAP, which showed no linear term ($p < 0.05$). In short, temperature (X_3) followed by the molar ratio (X_1) was the most important linear term. Concerning the interaction terms, some responses exhibited a high number of significant terms such as FRAP, DPPH and chlorogenic acid. Meanwhile, TPC had no significant interaction term. According to the number of significant interaction terms, water content time (X_2X_4) interaction was the most relevant interaction for most responses, followed by molar ratio water content (X_1X_2). Furthermore, the quadratic terms revealed an interesting result, where the molar ratio (X_1X_1) was the most significant for most responses.

The final polynomial equations are given in terms of the coded factors for all responses as follows (Equations 3-13):

$$\begin{aligned}\text{TPC mg/g} &= 24.04 - 2.73X_1 - 0.41X_2 + 4.3X_3 + 0.32X_4 \\ &\quad - 0.44X_{12} - 1.4X_{13} + 1.38X_{14} + 0.77X_{23} + 0.9X_{24} \\ &\quad + 0.2X_{34} - 0.08{X_1}^2 + 1.24{X_2}^2 - 0.41{X_3}^2 - 1.66{X_4}^2 \end{aligned} \quad (3)$$

$$\begin{aligned}\text{TFC mg/g} &= 0.94 + 0.22X_1 - 0.46X_2 + 0.12X_3 - 0.37X_4 \\ &\quad - 0.03X_{12} + 0.23X_{13} + 0.12X_{14} - 0.02X_{23} + 0.44X_{24} \\ &\quad + 0.1X_{34} - 0.11{X_1}^2 + 0.16{X_2}^2 + 0.01{X_3}^2 - 0.1{X_4}^2 \end{aligned} \quad (4)$$

$$\begin{aligned}\text{DPPH mmol TE/g} &= 121.36 - 1.52X_1 + 2.1X_2 - 12.57X_3 + 12.34X_4 \\ &\quad + 6.31X_{12} + 7.1X_{13} - 6.97X_{14} - 4.4X_{23} - 12.5X_{24} \\ &\quad - 7.17X_{34} - 6.16{X_1}^2 + 1.03{X_2}^2 - 8{X_3}^2 - 11.63{X_4}^2 \end{aligned} \quad (5)$$

$$\text{FRAP mmol ISE/g} = 155.06 - 0.92X_1 - 0.67X_2 - 2.58X_3 - 0.36X_4 - 10.47X_{12} + 8.68X_{13} - 6.42X_{14} - 0.31X_{23} + 1.87X_{24} + 5.73X_{34} - 2.73X_1^2 - 14.25X_2^2 + 4.9X_3^2 - 2.32X_4^2 \quad (6)$$

$$\text{Gallic acid mg/kg} = 266.85 + 6.75X_1 + 18.52X_2 + 54.88X_3 + 19.48X_4 - 1.28X_{12} + 1.47X_{13} - 4.46X_{14} + 7.24X_{23} + 10.87X_{24} + 15.37X_{34} - 20.03X_1^2 + 6.79X_2^2 + 8.67X_3^2 + 1.78X_4^2 \quad (7)$$

$$\text{Chlorogenic acid mg/kg} = 41.07 + 0.94X_1 + 0.09X_2 - 0.27X_3 + 0.25X_4 - 0.68X_{12} + 0.07X_{13} + 1X_{14} - 0.33X_{23} + 0.48X_{24} - 1.32X_{34} - 0.19X_1^2 - 0.43X_2^2 - 0.63X_3^2 + 0.49X_4^2 \quad (8)$$

$$\text{Vanillic acid mg/kg} = 75.75 + 0.51X_1 + 2.3X_2 - 0.24X_3 + 0X_4 + 6.01X_{12} + 1.04X_{13} + 0.51X_{14} - 0.06X_{23} + 3.33X_{24} - 1.2X_{34} - 1.21X_1^2 - 4.58X_2^2 - 0.59X_3^2 - 2.91X_4^2 \quad (9)$$

$$\text{Epicatechin mg/kg} = 285.08 + 14.13X_1 + 3.59X_2 - 2.52X_3 - 7.45X_4 + 3.16X_{12} + 1.29X_{13} + 3.85X_{14} - 9.39X_{23} + 2.43X_{24} - 2.58X_{34} - 12.74X_1^2 + 0.55X_2^2 - 12.76X_3^2 - 3.16X_4^2 \quad (10)$$

$$\text{Syringic acid mg/kg} = 21.59 + 1.49X_1 - 0.18X_2 + 2.49X_3 + 0.42X_4 + 0.2X_{12} + 0.16X_{13} + 0.24X_{14} + 0.55X_{23} + 1X_{24} + 0.56X_{34} - 0.72X_1^2 + 0.72X_2^2 + 0.23X_3^2 - 0.02X_4^2 \quad (11)$$

$$\text{Quercetin} - 3 - \text{glucoside mg/kg} = 75.4 + 4.17X_1 - 5.34X_2 + 4.69X_3 + 0.12X_4 - 0.03X_{12} + 2.17X_{13} + 1.43X_{14} - 1.12X_{23} + 5.83X_{24} + 0.75X_{34} + 3.22X_1^2 + 4.27X_2^2 - 4.69X_3^2 - 0.36X_4^2 \quad (12)$$

$$\text{Salicylic acid mg/kg} = 322.28 + 25.53X_1 - 5.38X_2 + 12.27X_3 + 13.01X_4 - 8.49X_{12} - 6.77X_{13} - 18.62X_{14} + 11.74X_{23} + 5.45X_{24} + 1.84X_{34} - 16.84X_1^2 + 5.73X_2^2 + 9.24X_3^2 + 11.89X_4^2 \quad (13)$$

The polynomial equations are used for the generation of perturbation plots (Fig. 2a–k). The generated plots are created according to fixed actual factors; 3.5 of molar ratio, 30% of water content, at 58 °C and 25 min of extraction time. The obtained curves took downward and upward linear and parabolic curves. The comparison between factors' effects was realized based on the angle between the curves and the horizontal reference line.

According to the plots (Fig. 2a, e, i) and comparing the extraction factors, the temperature was the most effective factor for the extraction of TPC, gallic acid and syringic acid which have demonstrated a positive linear increase at low temperatures (≈ 40 °C) to high temperatures (≈ 70 °C). Oppositely, some responses such as DPPH (Fig. 2a) and epicatechin (Fig. 1h) were negatively affected by the increase in temperature.

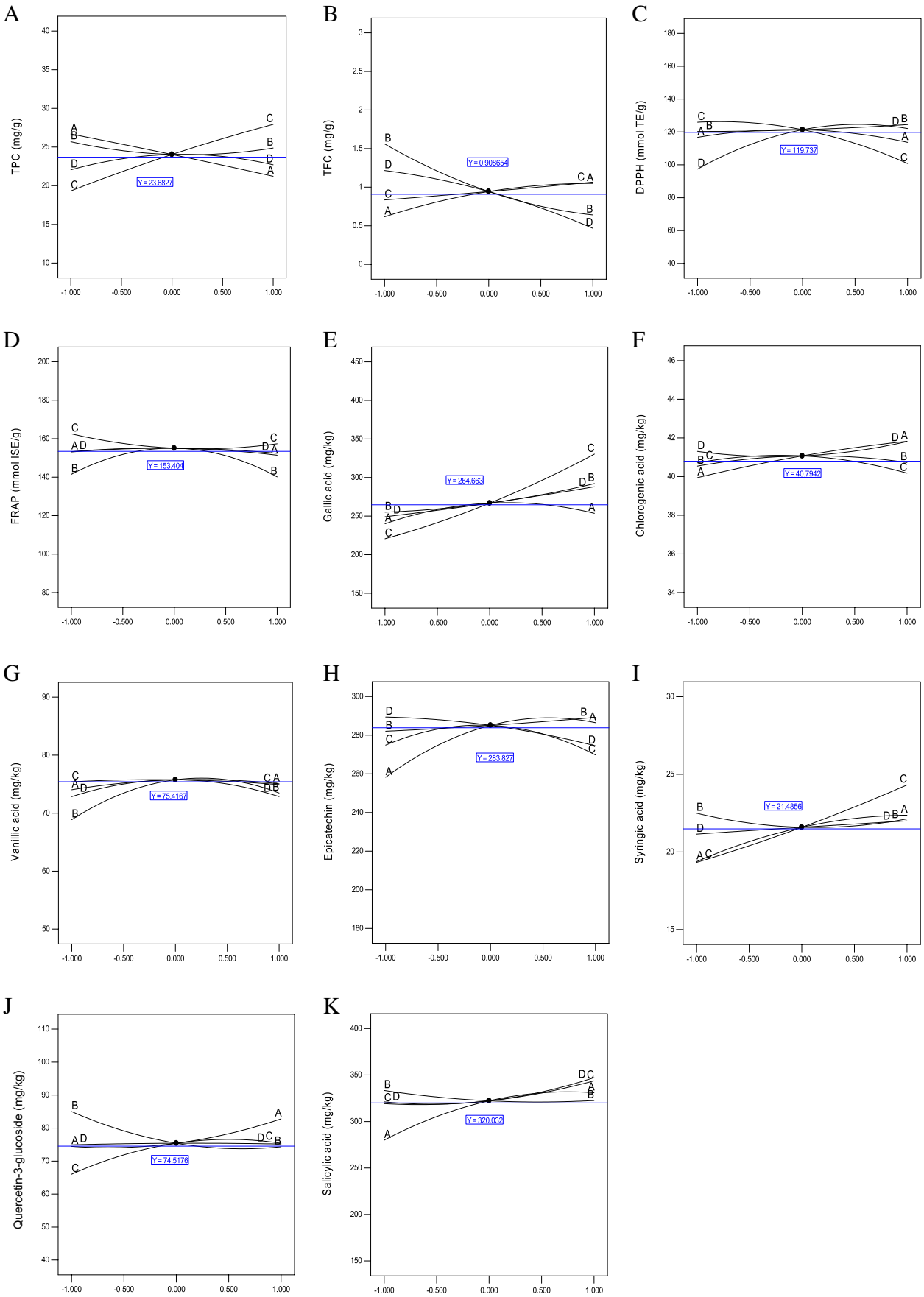
Moreover, the molar ratio factor (A) has effects on many responses. Some of them (TFC, chlorogenic acid, quercetin-3-glucoside) presented positive effects by increasing the molar ratio beyond 3.5 (Fig. 2b, f, j). Also, while a variation of molar ratio did not influence some responses, the low values affected negatively the extraction of some phenolic compounds such as epicatechin, syringic acid and salicylic acid (Fig. 2h, i, k). On contrary, the increase in molar ratio has decreased the total phenolic compounds recovery (Fig. 2a).

Water content variation (B) was also an important factor to take into consideration. Low values of water were a significant factor to increase values for many responses such as TFC, syringic acid, quercetin-3-glucoside and salicylic acid (Fig. 2b, i, j, k). Meanwhile, the most response showed less and insignificant effect at high water content values.

Similarly, the time factor was less effective for extraction efficiency, except for chlorogenic acid and salicylic acid at a long extraction time (Fig. 2f, k). However, a long extraction time has a negative effect on the total flavonoids and epicatechin (Fig. 2b, h).

3.2.1 Multi-response of RSM

The application of the desirability function of RSM generated the optimum conditions which were 1:4.35 for molar ratio, 20.68% for water content, 75 °C for temperature and 21.23 min for extraction time. Under these conditions, theoretical values were 26.12 mg GAE/g, 2.10 mg ECE/g, 95.15 mmol TE/g, 154.65 mmol ISE/g, 303.07 mg/kg, 41.32 mg/kg, 67.27 mg/kg, 282.04 mg/kg, 25.14 mg/kg, 91.16 mg/kg and 354.55 mg/kg for TPC, TFC, DPPH, FRAP, gallic acid, chlorogenic acid, vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside and salicylic acid, respectively. Further experimentations were conducted in triplicate under the same optimum conditions to verify the theoretical values. The results were 27.02 ± 1.22 mg GAE/g, 2.02 ± 0.09 mg ECE/g, 97.25 ± 3.51 mmol TE/g, 152.65 ± 5.02 mmol ISE/g, 307.01 ± 7.12 mg/kg, 45.02 ± 2.03 mg/kg, 69.07 ± 3.10 mg/kg, 281.84 ± 2.98 mg/kg, 26.24 ± 1.01 mg/kg, 92.36 ± 4.01 mg/kg and 352.95 ± 5.95 mg/kg for TPC, TFC, DPPH, FRAP, gallic acid, chlorogenic acid, vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside and salicylic acid, respectively. As can be evidenced, the predicted and experimental values were found very close. Therefore, the RSM can be said


A
TPC (mg/g)
Y = 23.6827
B
TFC (mg/g)
Y = 0.908654
C
DPPH (mmol TE/g)
Y = 119.737
D
FRAP (mmol ISE/g)
Y = 153.404
E
Gallic acid (mg/kg)
Y = 264.663
F
Chlorogenic acid (mg/kg)
Y = 40.7942
G
Vanillic acid (mg/kg)
Y = 75.4167
H
Epicatechin (mg/kg)
Y = 283.827
I
Syringic acid (mg/kg)
Y = 21.4856
J
Quercetin-3-glucoside (mg/kg)
Y = 74.5176
K
Salicylic acid (mg/kg)
Y = 320.032

◂**Fig. 2** Perturbations plots showing the effects of the studied factors (A: molar ratio, B: water content, C: temperature, D: time) on the different responses (**a** TPC, **b** TFC, **c** DPPH, **d** FRAP, **e** gallic acid, **f** chlorogenic acid, **g**: vanillic acid, **h** epicatechin, **i** syringic acid, **j** quercetin-3-glucoside, **k** salicylic acid)

reliable and reproducible to investigate the optimization of phenolic compounds and antioxidant activity of bitter melon.

3.3 In vitro bioaccessibility of bitter melon phenolics

The bioaccessibility of bitter melon leaf phenolic compounds was determined and are presented in Table 5. The most bioaccessible compound was vanillic acid (105.00 ± 2.52%) followed by salicylic acid, chlorogenic acid, syringic acid, gallic acid, epicatechin and quercetin-3-glucoside. These results suggest that bitter melon leaves could provide highly bioaccessible (> 80%) vanillic acid, salicylic acid and chlorogenic acid. Bitter melon leaves' vanillic acid bioaccessibility is higher than that reported from garlic (69%) [36], carob pod (87–95%) [37], umbu-cajá and (45.88%) and mangaba (57.73%) [38]. In addition, bitter melon leaves' salicylic acid bioaccessibility is greater than that reported from umbu-cajá (21.60%), siriguela (13.08%) and mangaba (18.27%) [38]. Furthermore, bitter melon leaves' salicylic acid bioaccessibility is greater than that reported from canned tomatoes (25.8%) [39]. Interestingly, we found in the present study high bioaccessibility bitter melon phenolic compounds, suggesting that the NADES with 20% water exerted a protective effect on these phenolic compounds in the gastrointestinal tract. These results are supported by those of Zannou et al. [40] who reported the in vitro bioavailability of the phenolic compounds of *E. amoenum* to be 82.99 ± 2.51%, 53.09 ± 4.78%, 33.84 ± 0.06% and 18.20 ± 1.06% for gallic acid, vanillic acid, epicatechin and chlorogenic acid extracted with choline chloride-glycerol-based NADES, respectively. This protective effect of NADES with 20% water is due to the strong hydrogen bonding formed between NADES with 20% water and phenolic compounds [11, 40]. In addition, the previous studies demonstrated that the solubilization properties of different eutectic mixtures increase the bioavailability of bioactive compounds [41–43].

As seen in the results (Table 5), the in vitro bioaccessibility of phenolic compounds differ significantly ($p < 0.05$). Differences in bioaccessibility are due to variations in the liberation of the phenolic compounds caused by the phenolic-matrix interaction [44]. The free phenolic compounds that are present as phenolic aglycones and conjugated phenolics are typically easily released from the matrix in the digestive fluid and absorbed. On the other hand, the phenolic compounds that are covalently bound to indigestible matrices like polysaccharides and structural proteins are poorly released into the digestive juice to be absorbed [45]. Thus, these phenolic compounds have low bioaccessibility. In addition, the chemical structure of phenolic compound can influence its bioaccessibility. Phenolic compounds bound to sugars as β-glycosides, such as quercetin-3-glucoside (Fig. 1), are poorly absorbable; only aglycones can pass through the gut wall [46]. In fact, flavonoid glycosides can only be absorbed after their hydrolysis by colon microorganisms. This explains why quercetin-3-glucoside had a very low bioaccessibility.

Table 5 Bioaccessibility percentage of bitter melon phenolic compounds

Phenolic compounds	Bioaccessibility (%)
Gallic acid	22.10 ± 0.62^{e}
Chlorogenic acid	80.50 ± 5.23^{c}
Vanillic acid	105.00 ± 2.52^{a}
Epicatechin	16.95 ± 0.06^{e}
Syringic acid	55.46 ± 0.26^{d}
Quercetin-3-glucoside	4.35 ± 0.51^{f}
Salicylic acid	97.11 ± 2.71^{b}

Lowercase letters a–f in the same column mean significant difference at $p < 0.05$

4 Conclusions

In the present study, the ability of NADES with 20% water (CHAC) to enhance the extraction of phenolic compounds from bitter melon leaves was investigated. The results indicated that CHAC showed higher yields of total phenolic content, total flavonoid content, individual phenolic compounds and antioxidant activity compared to conventional solvents (water, methanol and ethanol). Surface response optimization was applied to determine the optimum extraction conditions (molar ratio of solvent, water content, extraction temperature and time) for improved extraction of phenolic compounds. The optimum conditions were found as 1:4.35 for molar ratio, 20.68% for water content, 75 °C for temperature and 21.23 min for extraction time. Under these conditions, the experimental results were 27.02 ± 1.22 mg GAE/g, 2.02 ± 0.09 mg ECE/g, 97.25 ± 3.51 mmol TE/g, 152.65 ± 5.02 mmol ISE/g, 307.01 ± 7.12 mg/kg, 45.02 ± 2.03 mg/kg, 69.07 ± 3.10 mg/kg, 281.84 ± 2.98 mg/kg, 26.24 ± 1.01 mg/kg, 92.36 ± 4.01 mg/kg and 352.95 ± 5.95 mg/kg for TPC, TFC, DPPH, FRAP, gallic acid, chlorogenic acid, vanillic acid, epicatechin, syringic acid, quercetin-3-glucoside and salicylic acid, respectively. Moreover, the bioaccessibility of the phenolic compounds of the extract obtained under optimum conditions was determined. The results revealed that bitter melon leaves can be

considered a source of highly bioaccessible vanillic acid, salicylic acid and chlorogenic acid. CHAC can be used as a green extracting solvent for enhanced extraction of phenolic compounds from plant materials.

Author contribution Oscar Zannou: conceptualization, data curation, methodology, visualization, writing — original draft, writing — review and editing.

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Mohamed Ghellam: conceptualization, data curation, methodology, visualization, writing — original draft, writing — review and editing.

Ali Ali Redha: data curation, funding acquisition, validation, visualization, writing — original draft, writing — review and editing.

Ilkay Koca: conceptualization, data curation, funding acquisition, methodology, project administration, supervision, validation, visualization, writing — original draft, writing — review and editing.

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Declarations

Conflict of interest The authors declare no competing interests.

References

1. Grover JK, Yadav SP (2004) Pharmacological actions and potential uses of Momordica charantia: a review. J Ethnopharmacol 93:123–132. https://doi.org/10.1016/j.jep.2004.03.035
2. Zhang F, Lin L, Xie J (2016) A mini-review of chemical and biological properties of polysaccharides from Momordica charantia. Int J Biol Macromol 92:246–253. https://doi.org/10.1016/j.ijbiomac.2016.06.101
3. Virdi J, Sivakami S, Shahani S et al (2003) Antihyperglycemic effects of three extracts from Momordica charantia. J Ethnopharmacol 88:107–111. https://doi.org/10.1016/S0378-8741(03)00184-3
4. Kubola J, Siriamornpun S (2008) Phenolic contents and antioxidant activities of bitter gourd (Momordica charantia L.) leaf, stem and fruit fraction extracts in vitro. Food Chem 110:881–890. https://doi.org/10.1016/j.foodchem.2008.02.076
5. Mada SB, Garba A, Mohammed HA et al (2013) Antimicrobial activity and phytochemical screening of aqueous and ethanol extracts of Momordica charantia. L leaves 7:579–586
6. Bakare RI, Magbagbeola OA, Akinwande AI, Okunowo OW (2010) Nutritional and chemical evaluation of Momordica charantia. J Med Plants Res 4:2189–2193
7. LingWang BGC, Ya J et al (2008) Antifeedant activity and active ingredients against Plutella xylostella from Momordica charantia leaves. Agric Sci China 7:1466–1473. https://doi.org/10.1016/S1671-2927(08)60404-6
8. Hwang ES (2018) Comparison of antioxidant capacity and α -glucosidase inhibitory activity between bitter melon (Momordica charanti) fruit and leaf extract. Asian Pac J Trop Biomed 8:189–193. https://doi.org/10.4103/2221-1691.231280
9. Uysal S, Cvetanović A, Zengin G et al (2019) Optimization of maceration conditions for improving the extraction of phenolic compounds and antioxidant effects of Momordica charantia L. leaves through response surface methodology (RSM) and artificial neural networks (ANNs). Anal Lett 52:2150–2163. https://doi.org/10.1080/00032719.2019.1599007
10. Ali Redha A (2021) Review on extraction of phenolic compounds from natural sources using green deep eutectic solvents. J Agric Food Chem 69:878–912. https://doi.org/10.1021/acs.jafc.0c06641
11. Dai Y, van Spronsen J, Witkamp GJ et al (2013) Natural deep eutectic solvents as new potential media for green technology. Anal Chim Acta 766:61–68. https://doi.org/10.1016/j.aca.2012.12.019
12. Shakirova F, Shishov A, Bulatov A (2022) Hydrolysis of triglycerides in milk to provide fatty acids as precursors in the formation of deep eutectic solvent for extraction of polycyclic aromatic hydrocarbons. Talanta 237:122968. https://doi.org/10.1016/j.talanta.2021.122968
13. Sereshti H, Seraj M, Soltani S et al (2022) Development of a sustainable dispersive liquid–liquid microextraction based on novel hydrophobic and hydrophilic natural deep eutectic solvents for the analysis of multiclass pesticides in water. Microchem J 175:107226. https://doi.org/10.1016/j.microc.2022.107226
14. Dai Y, Verpoorte R, Choi YH (2014) Natural deep eutectic solvents providing enhanced stability of natural colorants from safflower (Carthamus tinctorius). Food Chem 159:116–121. https://doi.org/10.1016/j.foodchem.2014.02.155
15. Zannou O, Pashazadeh H, Ghellam M, Ibrahim SA, Koca I (2022) Extraction of Anthocyanins from Borage (Echium amoenum) flowers using choline chloride and a glycerol-based, deep eutectic solvent: optimization, antioxidant activity, and in vitro bioavailability. Molecules 27(1):134. https://doi.org/10.3390/molecules27010134
16. Ali MC, Chen J, Zhang H et al (2019) Effective extraction of flavonoids from Lycium barbarum L. fruits by deep eutectic solvents-based ultrasound-assisted extraction. Talanta 203:16–22. https://doi.org/10.1016/j.talanta.2019.05.012
17. Liu Y, Zhe W, Zhang R, et al (2022) Ultrasonic-assisted extraction of polyphenolic compounds from Paederia scandens (Lour.) Merr. using deep eutectic solvent: optimization, identification, and comparison with traditional methods. Ultrason Sonochem 86:106005. https://doi.org/10.1016/j.ultsonch.2022.106005
18. Liu Y, Kong KW, Wu DT et al (2022) Pomegranate peel-derived punicalagin: ultrasonic-assisted extraction, purification, and its α-glucosidase inhibitory mechanism. Food Chem 374:131635. https://doi.org/10.1016/j.foodchem.2021.131635
19. Chanioti S, Tzia C (2018) Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. Innov Food Sci Emerg Technol 48:228–239. https://doi.org/10.1016/j.ifset.2018.07.001
20. Daghaghele S, Kiasat AR, SafieddinArdebili SM, Mirzajani R (2021) Intensification of extraction of antioxidant compounds from Moringa oleifera leaves using ultrasound-assisted

approach: BBD-RSM Design. Int J Fruit Sci 21:693–705. https://doi.org/10.1080/15538362.2021.1926396
21. Chanioti S, Tzia C (2017) Optimization of ultrasound-assisted extraction of oil from olive pomace using response surface technology: oil recovery, unsaponifiable matter, total phenol content and antioxidant activity. LWT - Food Sci Technol 79:178–189. https://doi.org/10.1016/j.lwt.2017.01.029
22. Jerman T, Trebše P, MozetičVodopivec B (2010) Ultrasound-assisted solid liquid extraction (USLE) of olive fruit (Olea europaea) phenolic compounds. Food Chem 123:175–182. https://doi.org/10.1016/j.foodchem.2010.04.006
23. Rodriguez N, Van Den Bruinhorst A, Kollau LJBM et al (2019) Degradation of deep-eutectic solvents based on choline chloride and carboxylic acids. ACS Sustain Chem Eng 7:11521–11528. https://doi.org/10.1021/acssuschemeng.9b01378
24. Florindo C, Oliveira FS, Rebelo LPN et al (2014) Insights into the synthesis and properties of deep eutectic solvents based on cholinium chloride and carboxylic acids. ACS Sustain Chem Eng 2:2416–2425. https://doi.org/10.1021/sc500439w
25. Nguyen HTL, Kasapis S, Mantri N (2021) Physicochemical properties and effects of honeys on key biomarkers of oxidative stress and cholesterol homeostasis in hepg2 cells. Nutrients 13:1–19. https://doi.org/10.3390/nu13010151
26. Zannou O, Koca I (2020) Optimization and stabilization of the antioxidant properties from Alkanet (Alkanna tinctoria) with natural deep eutectic solvents. Arab J Chem 13:6437–6450. https://doi.org/10.1016/j.arabjc.2020.06.002
27. Bosiljkov T, Dujmić F, CvjetkoBubalo M et al (2017) Natural deep eutectic solvents and ultrasound-assisted extraction: Green approaches for extraction of wine lees anthocyanins. Food Bioprod Process 102:195–203. https://doi.org/10.1016/j.fbp.2016.12.005
28. Salvia-Trujillo L, McClements DJ (2016) Enhancement of lycopene bioaccessibility from tomato juice using excipient emulsions: influence of lipid droplet size. Food Chem 210:295–304. https://doi.org/10.1016/j.foodchem.2016.04.125
29. Zannou O, Koca I (2022) Greener extraction of anthocyanins and antioxidant activity from blackberry (Rubus spp) using natural deep eutectic solvents. Lwt 158:113184. https://doi.org/10.1016/j.lwt.2022.113184
30. de Almeida Pontes PV, Ayumi Shiwaku I, Maximo GJ, Caldas Batista EA (2021) Choline chloride-based deep eutectic solvents as potential solvent for extraction of phenolic compounds from olive leaves: extraction optimization and solvent characterization. Food Chem 352https://doi.org/10.1016/j.foodchem.2021.129346
31. Tan SP, Stathopoulos C, Parks S, Roach P (2014) An optimised aqueous extract of phenolic compounds from bitter melon with high antioxidant capacity. Antioxidants 3:814–829. https://doi.org/10.3390/antiox3040814
32. Huang WC, Tsai TH, Huang CJ et al (2015) Inhibitory effects of wild bitter melon leaf extract on Propionibacterium acnes-induced skin inflammation in mice and cytokine production in vitro. Food Funct 6:2550–2560. https://doi.org/10.1039/c5fo00550g
33. Tsai T-H, Huang C-J, Wu W-H et al (2014) Antioxidant, cell-protective, and anti-melanogenic activities of leaf extracts from wild bitter melon (Momordica charantia Linn. var. abbreviata Ser.) cultivars. Bot Stud 55:1–11. https://doi.org/10.1186/s40529-014-0078-y
34. Lestari P, Katrin R, Mahayasih PGMW (2017) Inhibition activity of angiotensin converting enzyme (ACE) and determination of total phenolic and flavonoid compound from bitter melon leaves (Momordica charantia L.). Pharmacogn J 9:252–256. https://doi.org/10.5530/pj.2017.2.43
35. Vieira V, Prieto MA, Barros L et al (2018) Enhanced extraction of phenolic compounds using choline chloride based deep eutectic solvents from Juglans regia L. Ind Crops Prod 115:261–271. https://doi.org/10.1016/j.indcrop.2018.02.029
36. Moreno-Ortega A, Pereira-Caro G, Ordóñez JL, Moreno-Rojas R, Ortíz-Somovilla V, Moreno-Rojas JM (2020) Bioaccessibility of bioactive compounds of 'fresh garlic' and 'black garlic' through in vitro gastrointestinal digestion. Foods 9(11):1582. https://doi.org/10.3390/foods9111582
37. Frühbauerová M, Červenka L, Hájek T, et al (2022) Bioaccessibility of phenolics from carob (Ceratonia siliqua L.) pod powder prepared by cryogenic and vibratory grinding. Food Chem 377 https://doi.org/10.1016/j.foodchem.2021.131968
38. Dutra RLT, Dantas AM, de Marques DA et al (2017) Bioaccessibility and antioxidant activity of phenolic compounds in frozen pulps of Brazilian exotic fruits exposed to simulated gastrointestinal conditions. Food Res Int 100:650–657. https://doi.org/10.1016/j.foodres.2017.07.047
39. Izzo L, Castaldo L, Lombardi S, Gaspari A, Grosso M, Ritieni A (2022) Bioaccessibility and antioxidant capacity of bioactive compounds from various typologies of canned tomatoes. Front Nutr 9:849163. https://doi.org/10.3389/fnut.2022.849163
40. Zannou O, Pashazadeh H, Ghellam M et al (2022) Appraisal of phenolic compounds, antioxidant activity and in vitro gastrointestinal digestion of borage (Echium amoenum) flowers using natural deep eutectic solvent (NADES). Biomass Convers Biorefinery. https://doi.org/10.1007/s13399-022-02739-z
41. Faggian M, Sut S, Perissutti B et al (2016) Natural Deep Eutectic Solvents (NADES) as a tool for bioavailability improvement: pharmacokinetics of rutin dissolved in proline/glycine after oral administration in rats: Possible application in nutraceuticals. Molecules 21:1–11. https://doi.org/10.3390/molecules21111531
42. da Silva DT, Smaniotto FA, Costa IF, et al (2021) Natural deep eutectic solvent (NADES): a strategy to improve the bioavailability of blueberry phenolic compounds in a ready-to-use extract. Food Chem 364https://doi.org/10.1016/j.foodchem.2021.130370
43. Sut S, Faggian M, Baldan V et al (2017) Natural Deep Eutectic Solvents (NADES) to enhance berberine absorption: an in vivo pharmacokinetic study. Molecules 22:1–11. https://doi.org/10.3390/molecules22111921
44 Shahidi F, Peng H (2018) Bioaccessibility and bioavailability of phenolic compounds. J Food Bioact 4:11–68. https://doi.org/10.31665/jfb.2018.4162
45. Shahidi F, Ambigaipalan P (2015) Phenolics and polyphenolics in foods, beverages and spices: antioxidant activity and health effects - a review. J Funct Foods 18:820–897. https://doi.org/10.1016/j.jff.2015.06.018
46. Gutiérrez-Grijalva EP, Ambriz-Pére DL, Leyva-López N et al (2016) Review: dietary phenolic compounds, health benefits and bioaccessibility. Arch Latinoam Nutr 66:87–100