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Green extraction of arabica coffee cherry husk using a deep eutectic solvent

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ABSTRACT: Dried coffee cherry husk (DCCH) waste, obtained from arabica coffee processing, contains many valuable phytochemical substances. Extraction of phytochemicals causes value-addition to this waste. Organic solvent extraction is one of the most effective methods. However, the "green chemistry" concept has been considered to reduce environmental contamination and chemical-related effects on human health. Natural deep eutectic solvents (DESs) were used as an alternative solvent for the extraction of polyphenols from DCCH. In this work, the choline chloride-citric acid deep eutectic solvent (ChCA) was produced from 1:2 mole ratio of choline chloride (ChCl) to citric acid (CA) along with ultrasound-assisted extraction at 25 °C for 60 min. The ratio of water combined with ChCA ranged from 20–50% w/w. Functional groups and viscosity of ChCA and ChCA mixed with water contents were identified by Fourier transform infrared spectroscopy (FTIR) and viscosity analysis, respectively. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) of the DCCH crude extracts from DES were examined. Extraction using 40% w/w water/ChCA produced higher levels of TPC, TFC, and AA than other conditions at 27.00 ± 1.18 mg GAE/g DCCH, 17.19 ± 0.12 mg CE/g DCCH, and 11.24 ± 0.32 mg AAE/g DCCH, respectively. The phytochemicals extracted from DCCH were analyzed by ultra-high-performance liquid chromatography with the mass quadrupole time of flight mass spectrometer (LC-QTOF) in positive and negative ionization modes. A total of 718 compounds significantly differed from other sample groups. The designed DES extraction can be successfully used to produce high-added-value phytochemicals from DCCH.

KEYWORDS: dried coffee cherry husks, natural deep eutectic solvent, antioxidant activity, total phenolic content, total flavonoid content

INTRODUCTION

Coffee has always been one of the most consumed beverages in the world. Consequently, the coffee industry can cause tremendous amounts of by-products and residues to happen. Coffee cherry husks (CCHs) comprising outer skin, pulp, and parchment are the main residue obtained during the processing of coffee cherries by dry process [1, 2]. Since it contains bioactive compounds such as polyphenols including chlorogenic (CGA), protocatechuic (PCA), and gallic acids (GA) which are the main components in CCHs [2–5], it has been considered raw materials for extraction of bioactive compounds which could be applied for food, cosmetics, and medicinal products. The extracts of coffee husks are generally performed in water and organic solvents such as methanol [6], ethanol [7], and ethyl acetate [8] which can cause toxicity in the extract and potentially harm the extraction operator. To avoid the toxicity of organic solvents, using green solvent extraction has received much attention in recent years.

Various modern green technologies such as supercritical fluid extraction (SFE), pressurized hot water extraction (PHWE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) have been extensively employed as promising alternatives to conventional extraction (CE) methods. The separation of phenolic compounds from food materials using the SFE method has been reviewed [9, 10]. Supercritical fluids (SCF) are prominent solvents for this technique as their physical properties such as viscosity, density, polarity, and diffusivity can be dramatically adjusted by slightly changing pressure or temperature. SFE of wine grape seeds using CO_2 at critical temperature and pressure of 313 K and 35 MPa yielded higher efficient recovery of phenolic compounds when compared to conventional distillation [11]. However, the use of SFE with CO_2 for phenolic compounds in spent coffee grounds and coffee husks resulted in lower global extraction yield when compared to those obtained by soxhlet and ultrasound extraction in ethanol [12]. The application of PHWE for extracting phenolic compounds in plant matrices revealed high sensitivity and short extraction time [9, 13]. This method provided a high yield of chlorogenic acid, gentisic acid, and catechin extracts in bitter melon fruit and showed faster extraction time as compared to soxhlet extraction (SE) [14]. PHWE has been carried out using pressurized water at elevated temperatures which bring about significant changes in extractant

polarity and consequently increase solubility rates, leading to improvement in polyphenol extraction efficiency. Nevertheless, extraction temperature needs to be optimized to avoid the thermal degradation of phenolic compounds [9, 13]. UAE and MAE have become popular methods among other green technologies due to their lower operation temperature, less solvent, and energy consumption [15, 16]. Prevention of oxidative degradation for polyphenols during the extraction process can be achieved by operating these 2 techniques under a vacuum condition. The extraction of thermolabile phenolic compounds from red bayberry leaves using vacuum MAE with ethanol enhanced extraction efficiency with better recovery in less time than SE [9]. Optimization of the vacuum MAE method was performed to gain the maximum rate of extraction of polyphenols and flavonoids in orange pomace [17]. The result indicated the requirement of high microwave power and longtime intervals of operation for polyphenols maximum yield. In contrast to flavonoids, the optimum yield is achieved at low microwave power and moderate time owing to their chemical structure sensitivity. The production of bioactive natural extracts using UAE has been successfully carried out in Deep Eutectic Solvents (DESs). In a comparison of UAE, MAE, and CE for phenolics in red grape skin, it was found that choline chloride-based DES with oxalic acid and 25% of water addition yielded more effective extraction in the following order UAE > MAE > CE [18].

DESs are classified as green solvents which have been widely applied in dissolution and separation processes due to their high dissolution power and variation in physicochemical properties [19]. It has been revealed the compatibility of DESs with various applications related to extraction media of natural-based chemicals, nanomaterials synthesis, pharmaceutical, and medical products [20]. DESs are basically obtained by forming a quaternary ammonium salt that acts as a hydrogen bond acceptor (HBA) with a hydrogen bond donor (HBD) compound. Hydrogen bonding occurs through a halide ion and the hydrogen-donor moiety, the charge delocalization throughout the hydrogen bond between HBD and HBA consequently lowering the melting point of the mixture compared with their constituent pure components [21, 22]. Choline chloride (ChCl) is mostly used in DES synthesis among quaternary ammonium salts due to its low toxicity and biodegradability. Various types of compounds such as alcohol, sugar, polyols, amide, amino acid, and carboxylic acid have been widely used as HBD compositions [23–25]. Four target flavonoids, namely baicalin, wogonoside, baicalein, and wogonin, were successfully separated from *radix scutellariae* by MAE method in a series of 1:2 molar ratio of ChCl to different HBD: 1,4-butanediol, glycerol, ethylene glycol, citric acid, malic acid, lactic acid, glucose, sorbitol, sucrose, and maltose [26]. Extraction of polyphenols from spent filter coffee using ChCl to glycerol with the molar ratio of 1:3 coupled with UAE provided total polyphenols of 22.59 mg GAE/g [27]. The use of a 1:1 mole ratio of ChCl to lactic acid and 10% water addition along with UAE yielded 77.13 mg/g of curcuminoids in turmeric [28]. The physicochemical properties of DESs can be tunable by designing the type of HBDs and HBAs and their molar ratio [20]. For instance, a huge difference in viscosity was found when the mole ratio of ChCl to citric acid monohydrate (CA) changed from 1:1 to 1:2 [29]. In the previous study, the extraction efficiency of polyphenols in spent coffee grounds using ChCl-based DESs along with ultrasound-assisted extraction (UAE) indicated high extraction efficiency obtained from the DES prepared from a 1:2 mole ratio of ChCl:CA [30].

The present study aimed to investigate the optimal extraction of polyphenols from DCCHs using natural DESs composed of ChCl and CA. The chemical properties of crude extracts such as total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AA) were used for the investigation of the extraction efficiency of studied DESs and conventional solvents.

MATERIALS AND METHODS

Materials and chemicals

The CCHs used in this work were obtained from *Coffea arabica* L. and collected in 2019 from Doi Chang in Chiang Rai, Thailand. The solvents used for extraction included choline chloride (ChCl; purity ⩾ 98.0%; Loba Chemie, India), citric acid monohydrate (CA; purity ⩾ 99.5%; Sigma-Aldrich, USA), and methanol (Labscan, Thailand). All other chemicals used for measurements were of analytical grade: 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich), sodium nitrite and sodium carbonate anhydrous (Loba Chemie), aluminium chloride (QReC, New Zealand), Folin-Ciocalteu (Merck, Germany), and sodium hydroxide (Ajax Finechem, Australia). The standard chemicals used in this study were L(+)-ascorbic acid (POCH, Poland), (±)-catechin hydrate primary reference standard (Sigma-Aldrich), and gallic acid monohydrate standard (Fluka, Spain).

Synthesis of the DES mixture

DES mixture (ChCA) was prepared by complexation of a quaternary ammonium salt, choline chloride (ChCl) with a hydrogen-bond donor, citric acid monohydrate, with a molar ratio of 1:2 ChCl and CA). The binary mixture was heated at 80–100 °C and stirred briskly at about 200 rpm by a magnetic bar until a homogeneous solution was formed. ChCA with water addition was prepared by adding 20, 30, 40, and 50% water by weight of ChCA and designated code as ChCA-20W, ChCA-30W, ChCA-40w, and ChCA-50W, respectively.

Characterization of the DES

Hydrogen bonds existing in the DES were identified by the FTIR spectrometer (Nicolet iS50, Thermo Scientific, USA) with a built-in ATR over the wavenumber range 400–4000 cm^{-1}. The melting point of ChCA was determined using a DSC (DSC 3+, Mettler Toledo, Thailand) with a constant flow rate of nitrogen of 40 ml/min. In aluminum pans, ChCA was firmly enclosed. To establish the melting point, these analyses were carried out at temperatures ranging from −50 to 350 °C at a heating rate of 5 °C/min. The viscosity of the DES was determined using a rheometer (HAAKE RheoStress 1, Thermo Scientific, Australia) fitted with a parallel plate geometry with a 20 mm diameter and a 0.052 mm gap at 3 different temperatures (25, 35, and 45 °C). The measurements were carried out by equilibrating the sample temperature for 2 min before applying a shear rate of 1 s^{-1} for 30 s.

Ultrasonic-assisted extraction (UAE) of arabica coffee cherry husk

The collected CCHs were mixed into one sample for a representative sample of the extraction process using different solvents. Then, it was sun-dried before grinding into a fine powder. The 100 mg of DCCHs and 3 ml of prepared ChCA were combined in a 15 ml-microfuge tube and rapidly mixed by a vortex mixer. The extraction process was performed in a sonication bath (WUC-D03H, Daihan, Korea) with a 50% frequency (or 50% power level) at ambient temperature (25 °C) for 60 min. The supernatant was collected and made a 10-fold dilution with water for further chemical analysis. Apart from the ChCA, the extraction of coffee cherry husk was also performed in the DES with additional water: ChCA-20W, ChCA-30W, ChCA-40W, and ChCA-50W and typical solvents: MeOH and H_2O.

Assessment of antioxidant by DPPH assays (AA)

The DPPH radical-scavenging activity was determined according to a previously reported method with minor modification [31, 32]. The 1.0 ml of DPPH solution (0.3 mmol/l) was mixed with the 0.2 ml of the 100-fold dilution sample. The ascorbic acid used as a reference standard was performed in 5 series at concentrations of 0, 0.050, 0.100, 0.150, and 0.200 mM, respectively. The mixture was incubated for 30 min in the dark at ambient temperature. The absorbance of the mixture solution was monitored at 517 nm by a visible spectrometer (Ocean Optics USB4000 Spectrometer, USA). The deduction of the solvent background was performed using blank solutions prepared from various percentages of water in ChCA. The concentration was calculated and expressed as milligrams of ascorbic acid equivalents per gram of dried coffee cherry husk (DCCH) (mg AcAE/g DCCH).

Assessment of total phenolic content (TPC)

The extracts of coffee cherry husk were diluted 10-fold. The determination of TPC was adapted from the previous method [31, 32]. The 0.125 ml of the sample or the 0.2 ml of gallic acid standard (20, 40, 60, 80, and 100 ppm) was added into the 0.125 ml of 10% v/v Folin-Ciocalteu reagent in methanol. After that, the 1.25 ml of Na_2CO_3 was then transferred to the mixture and quickly adjusted with distilled water to get it up to 3.0 ml. The mixture was then incubated in the dark for 30 min at room temperature. Absorbance was detected at 725 nm by a visible spectrometer. The calibration curve was constructed with different concentrations of gallic acid as the standard, and ChCA mixed with water was used as blank solutions. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per gram of DCCH (mg GAE/g DCCH).

Assessment of total flavonoid content (TFC)

TFC was determined using aluminium chloride colorimetric assay [31, 32]. A 10-fold dilution of the extracts was performed before analysis. The 0.125 ml of the sample or 0.2 ml of catechin standard (20, 40, 60, 80, and 100 ppm) was added to the 0.1 ml of 0.55 M $AlCl_3$, then the 0.05 ml of 3 M $NaNO_2$ was added to the mixture. After that, 0.25 ml of 2.5 M NaOH was then added to the mixture and adjusted 1.475 ml distilled water to the mixture shortly. Subsequently, the mixture was incubated for 20 min in the dark at room temperature. Absorbance was determined at 510 nm by a visible spectrometer using ChCA mixed with different percentages of water as blank solutions. The concentration was calculated and expressed as milligrams of catechin equivalents per gram of DCCH (mg CE/g DCCH).

Qualitative analysis of a chemical profiling of the DCCH crude extract using LC-QTOF

The crude extract obtained from using DES with different percentages of water, MilliQ water, and methanol as extractant was filtered with a nylon filter (0.22 µm, 13 mm, Agilent Technologies, USA) and performed the qualitative analysis of a chemical profile by using an LCMS-UHPLC on an Agilent Infinity II 1290 instrument (Agilent Technologies) coupled to Agilent Technologies LCMS G6545B quadrupole time of flight mass spectrometer detector equipped with an electrospray ionization (ESI) source. The ESI conditions were carried out with the following parameters: the gas temperature at 300 °C, sheath gas temperature at 250 °C, sheath gas flow at 12 l/min, gas flow at 11 l/min, and Nebulizer at 45 psig.

The LCMS-UHPLC was run using an infinity Lab Poroshell 120 EC-C18 column (2.1 × 150 mm, i.d., 2.7 µm) reversible phase mode. The column temperature was kept constant at 35 °C when procedures were ongoing. The eluent was composed of 0.1%

formic acid in water (pump A) and 0.1% formic acid in acetonitrile (pump B).

The gradient mobile phase was controlled as follows: 0.00–1.00 min; 5% (Pump B), 1.00–13.00 min; 17% (Pump B), 13.00–25.00 min; 100% (Pump B), 25.00–33.00 min; 5% (Pump B), and 33.00 min, stop running. The injection volume was 1.00 µl with a flow rate of 0.400 ml/min, and the total running time was 33 min.

The mass peak lists extracted by a Masshunter workstation software B.08.00 (Agilent Technologies) in the DCCH crude extract obtained from various extractants were evaluated using one-way ANOVA (with $p < 0.05$) and post-hoc Tests (Fisher's LSD with $p < 0.05$), heat map, and principal component analysis via an online MetaboAnalyst program version 5.0. The statistical filters with 5% relative standard deviations and median normalization were performed before carrying out data analysis.

Data analysis

Chemical properties such as TPC, TFC, and AA were evaluated using one-way ANOVA with $p < 0.05$. Then, the correlation between the obtained chemical properties was studied by using Pearson's Correlation via MetaboAnalyst 5.0 online software.

RESULTS AND DISCUSSION

Melting points

DSC analysis reveals endothermic peaks at 305.08 °C and 153.08 °C corresponding to the melting points of ChCl and CA, respectively. The melting temperatures are consistent with those of ChCl [24] and CA [33]. The lower melting temperature of ChCA at 96.92 °C indicates liquid phase at temperatures lower than 100 °C which is generally found in DESs [34]. Deep eutectic melting point depression suggests the complexation of ChCl and hydrogen bond donor CA.

Viscosity

Viscosity is defined as internal friction to the flow caused by intermolecular interactions of medium components. This property plays an important role in all physical processes related to fluid movement and component dissolution [35]. The viscosity of the ChCA and its water addition was measured as a function of temperature and was listed in Table 1. The viscosity of the ChCA (1:2 molar ratio of choline chloride and citric acid monohydrate) at 25, 35, and 45 °C were 1236.69, 419.68, and 76.01 mPa s, respectively. Very high values of viscosity of ChCA were consistent with the reported range of DESs which can be 20 to more than 10^4 mPa s at 25 °C [36]. The viscosity of ChCl-ethylene glycol and ChCl-urea DESs with a 1:2 mole ratio at 25 °C was reported as 37.3 mPa s and 1571 mPa s, respectively [36]. A large range of viscosity for DESs involves many factors such as van der Waals forces, hydrogen bonding, electrostatic interactions, the molar ratio, and the nature of their single component [36, 37].

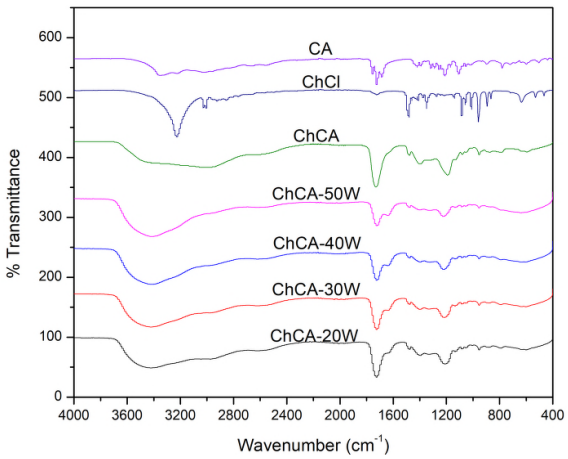

Fig. 1 FTIR spectra of ChCl and CA compared with ChCA, ChCA-20W, ChCA-30W, ChCA-40W, and ChCA-50W.

Although the increase in temperature can enhance the penetration of DESs in the extraction matrix, it does not always yield high extraction efficiency as it consumes more energy and can affect the heat-sensitive phytochemical compounds [23]. Apart from the temperature, water addition to the ChCA tremendously reduced the viscosity. Additional 20% to 50% (w/w) water causes a viscosity decrease as shown in Table 1, for example, at 25 °C from 165.52 ± 0.49 to 15.72 ± 0.48 Pa s, respectively. Water is therefore a good choice to use as a co-solvent for reducing the viscosity of ChCA. The ChCA mediums containing different water contents would be more suitable for the extraction of phytochemical compounds from plants [38]. In this work, increasing water content increased the deviation of data as shown in Table 1. Less reproducibility arose from the change in physicochemical properties which are particularly sensitive to the presence of moisture in the sample [36].

FTIR spectra

In order to trace the formation of hydrogen bond interactions in the DES (Table 2), FTIR spectra of the ChCA were compared with those of ChCl and CA (Fig. 1). ChCl spectrum revealed the presence of O—H stretching appears at 3227.06 cm^{-1}. The O—H in the carboxyl group on CA presents at 3228.27 cm^{-1}. In the ChCA spectrum, the peak shows broad stretching vibration at around 2750–3650 cm^{-1}. This reveals an occurrence of the O—H—Cl bond formed by hydroxyl groups on CA attracting to chlorine anion in ChCl [38]. Adding water into the DES solution with various amounts increased the transmittance percentage of O—H stretching at 3250–3500 cm^{-1} that resulted from O—H in water. However, when adding water from

Table 1 Average viscosity ($n = 3$) of ChCA and ChCA in the presence of water at different temperatures.

Solvent	Viscosity (Pa s)					
	at 25 °C		at 35 °C		at 45 °C	
	Mean ± SD	%RSD	Mean ± SD	%RSD	Mean ± SD	%RSD
ChCA	$1236.69 \times 10^3 \pm 1.82 \times 10^3$	0.15%	$419.68 \times 10^3 \pm 1.04 \times 10^3$	0.25%	$76.10 \times 10^3 \pm 0.62 \times 10^3$	0.82%
ChCA-20W	165.52 ± 0.49	0.30%	87.86 ± 1.26	1.43%	64.36 ± 0.69	1.06%
ChCA-30W	64.80 ± 0.89	1.37%	32.91 ± 0.18	0.56%	25.30 ± 0.52	2.07%
ChCA-40W	31.15 ± 0.56	1.81%	17.43 ± 0.27	1.58%	8.04 ± 0.08	1.02%
ChCA-50W	15.72 ± 0.48	3.08%	9.58 ± 0.62	6.49%	5.50 ± 0.12	2.26%

Table 2 Vibrational Band assignments and wavenumber exhibited by ChCl, CA, and DES.

Chemical	Wavenumber (cm^{-1})					
	O—H stretching	C=O bond	CH_2 bending	CH_3 bending	C—O stretching	C—N stretching
ChCl	3227.06	ND	1482.68	1349.65	ND	1085.57
CA	3228.27	1723.92	1418.37	ND	1170.01	ND
ChCA	2750.00–3650.00	1782.62	1477.98	1364.27	1190.22	1033.37
ChCA-20W	3420.74	1725.16	1477.93	1397.55	1208.5	1081.26
ChCA-30W	3417.43	1724.06	1478.10	1397.78	1216.82	1081.62
ChCA-40W	3412.96	1722.78	1478.13	1396.88	1220.27	1082.42
ChCA-50W	3413.04	1722.44	1478.17	1398.91	1221.4	1082.71

20 to 50% (w/w), hydrogen bond interactions in DES decreased as the wavenumber changed to lower levels, approximately 3400 cm^{-1}.

Evaluation of DES extraction efficiency

Phenolic acid and flavonoids are part of polyphenol compounds, which are natural antioxidants present in plant foods [39, 40]. The quantity of these compounds found in the extracts is therefore directly associated with their antioxidant potentials [3]. TPC, TFC, and AA were investigated to select the most efficient solvent. TPC and TFC in the DCCH extracted by ChCA with different water contents were examined and compared with the results obtained from typical solvents, H_2O, and MeOH (Table 3). The levels of TPC as mg GAE/g DCCH obtained from the solvent with 20, 30, 40, and 50% by weight water addition were found to be in a range of 13.04 ± 1.98 to 27.00 ± 1.18. This investigation supports the better extraction efficiency yielded from less viscous mediums containing the co-solvent, i.e., water. Nevertheless, the TPC level is highest when using 40% w/w water. The determination of TFC exhibits a similar pattern to the result obtained from the TPC study. The TFC results expressed as CE/g DCCH obtained from those studied % mixing water by weight were in a range of 6.45 ± 0.50 to 17.19 ± 0.12. Decreasing TPC and TFC levels were found in the extracts using 50% w/w water content. It was suggested that dilution with water caused a weakening of hydrogen bond interactions between ChCl and CA and could affect the dynamical properties such as fluidity, conductivity, and polarity of DESs [22, 29, 41]. The addition of water to DESs led to an increase in polarizability; higher water content can reduce the extraction efficiency of flavonoids [42, 43]. The higher polarity of solvents limited the solubility of extracted bioactive compounds and can lead to different levels of TPC, TFC, and AA. The study from this work suggested that most of the phenolic compounds present in DCCH are less polar in nature. In addition, the decrease in extraction yields of phenolic compounds in excessive dilution of DESs with water was explained in terms of decreasing in hydrogen bond interaction between DES components and bioactive compounds [44]. In this study, the use of 40% w/w water in the ChCA was the suggested condition for the extraction of the polyphenols in the DCCH. The TPC and TFC in the extracts obtained from using conventional solvents (methanol and water) were significantly lower than the levels obtained from the ChCA with water addition ($p < 0.05$). The DES solvent exhibits greater extraction efficiency and solubilization of a solute such as polyphenols than the liquid-liquid extraction method (methanol and water), which was due to the DES solvent exhibiting greater phase impact on the partition coefficient [45]. In addition, TPC and TFC obtained from the extraction had a strong correlation with Pearson's Correlation Coefficient (r) of 0.91. It could be implied that the extracted phenolic compounds tend to be a group of flavonoid compounds.

The results of the antioxidant activity of DCCH extracts measured from the DPPH assay are in accordance with the TPC and TFC levels. The antioxidant activities expressed as mg AcAE/g DCCH studied from the extracts using various % water addition were found

Table 3 Total phenolic content, total flavonoids, and antioxidant activity in the extracts of dried coffee cherry husk using different solvents ($n = 3$).

Solvent	Total phenolic (mg GAE/g DCCH)	Total plavonoid (mg CE/g DCCH)	Antioxidant activity (mg AcAE/g DCCH)
ChCA-20W	13.04 ± 1.98	6.45 ± 0.50	5.09 ± 0.17
ChCA-30W	20.78 ± 1.30	12.10 ± 0.48	9.76 ± 0.80
ChCA-40W	27.00 ± 1.18	17.19 ± 0.12	11.24 ± 0.32
ChCA-50W	18.34 ± 1.94	6.76 ± 0.22	4.61 ± 1.27
MeOH	9.49 ± 0.67	5.47 ± 0.25	1.16 ± 0.15
H_2O	7.63 ± 1.59	4.85 ± 0.40	3.77 ± 0.15

in the range of 4.61 ± 1.27 to 11.24 ± 0.32. The addition of 40% w/w H_2O to ChCA (ChCA-40W) displays the significantly highest antioxidant potential ($p < 0.05$) as it shows the highest values for the TPC and TFC. The strong correlations between AA and TPC, and AA and TFC were $r = 0.88$ and 0.92, respectively.

The application of ChCA-40W in this work shows 132% increase in the level of TPC as compared with the results reported by Yoo et al [30], where polyphenol compounds in spent coffee ground (SCGs) were extracted using a 1:2 mole ratio ChCl:CA irradiated with ultrasonic radiate at ambient temperature for 45 min. TFC level, however, increases only 7.4%. The different extraction efficiencies may be caused by the amounts of bioactive compounds existing in sources of materials. According to the report of Cangussu et al [46], the amounts of caffeine and chlorogenic acid in the CCH sample were 121.55 ± 0.18 mg/100 g and 618.10 ± 6.66 mg/100 g, respectively. While the SCG sample was found to have 1.45 mg/100 g of caffeine and 3.18 mg/100 g of chlorogenic acid, as reported by Bomfim et al [47]. Although the extracts are studied from different raw materials, it tends to indicate the applicability of the designed DES for extraction.

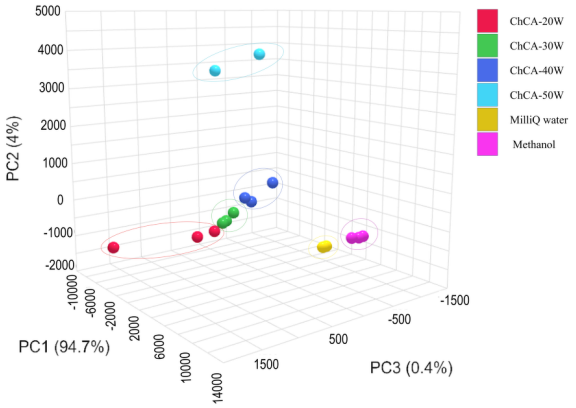

Fig. 2 The 3D-score plot between the selected principal components (PCs) obtained from the principal component analysis (PCA) according to the compound peak list (negative mode) of the DCCH crude extract extracted using different solvents: ChCA-20W (red dot), ChCA-30W (green dot), ChCA-40W (navy blue dot), ChCA-50W (light blue dot), Milli-Q water (yellow dot), and methanol (pink dot).

Qualitative analysis of a chemical profiling of the DCCH crude extract using LC-QTOF

The mass shown in the peak list of each sample was identified by databases such as Melin and Human Metabolome Database (HMDB). The statistical analysis was performed on both modes of the negative and positive mass peak lists. However, compounds analyzed by negative mode mass spectrometers show significant differences between sample groups. The compound lists with retention times identified by negative mode analysis in each crude sample that was extracted using various solvents are shown in the supplementary data. The average amounts of compounds found in crude samples after extraction with ChCA-20W, ChCA-30W, ChCA-40W, ChCA-50W, water, and methanol were 456 ± 2, 459 ± 5, 458 ± 4, 447 ± 5, 407 ± 11, and 1423 ± 246, respectively. It seems that increasing the water content in the ChCA from 20 to 40% shows a slight increase in the amount of found compounds until the water content reaches 50%. The amounts of compounds found in the crude sample extracted by water and methanol are significantly different from those obtained from using ChCA as a solvent. After the compound lists with retention times were evaluated by using the MetaboAnalyst online program, it is found that there are 718 compounds significantly different from other sample groups from the one-way ANOVA (with $p < 0.05$) and post-hoc tests (Fisher's LSD with $p < 0.05$). The top five masses (g/mol) that showed significantly different from each group are 366.0433 (unknown compound), 687.1844 (unknown compound), 817.1753 (unknown compound), 112.0161 (3-Methyl-2,5-furandione), and 367.1478 (N-Acetyl-6-O-L-fucosyl-D-Glucosamine). The principal component analysis was performed to examine the natural clustering of the compound peak list (negative mode) of the DCCH crude extract extracted using different solvents. The result was shown in Fig. 2. Compound peak lists were found to enable distinct clustering. The score plot suggested that 3 principal components were sufficient. The first principal com-

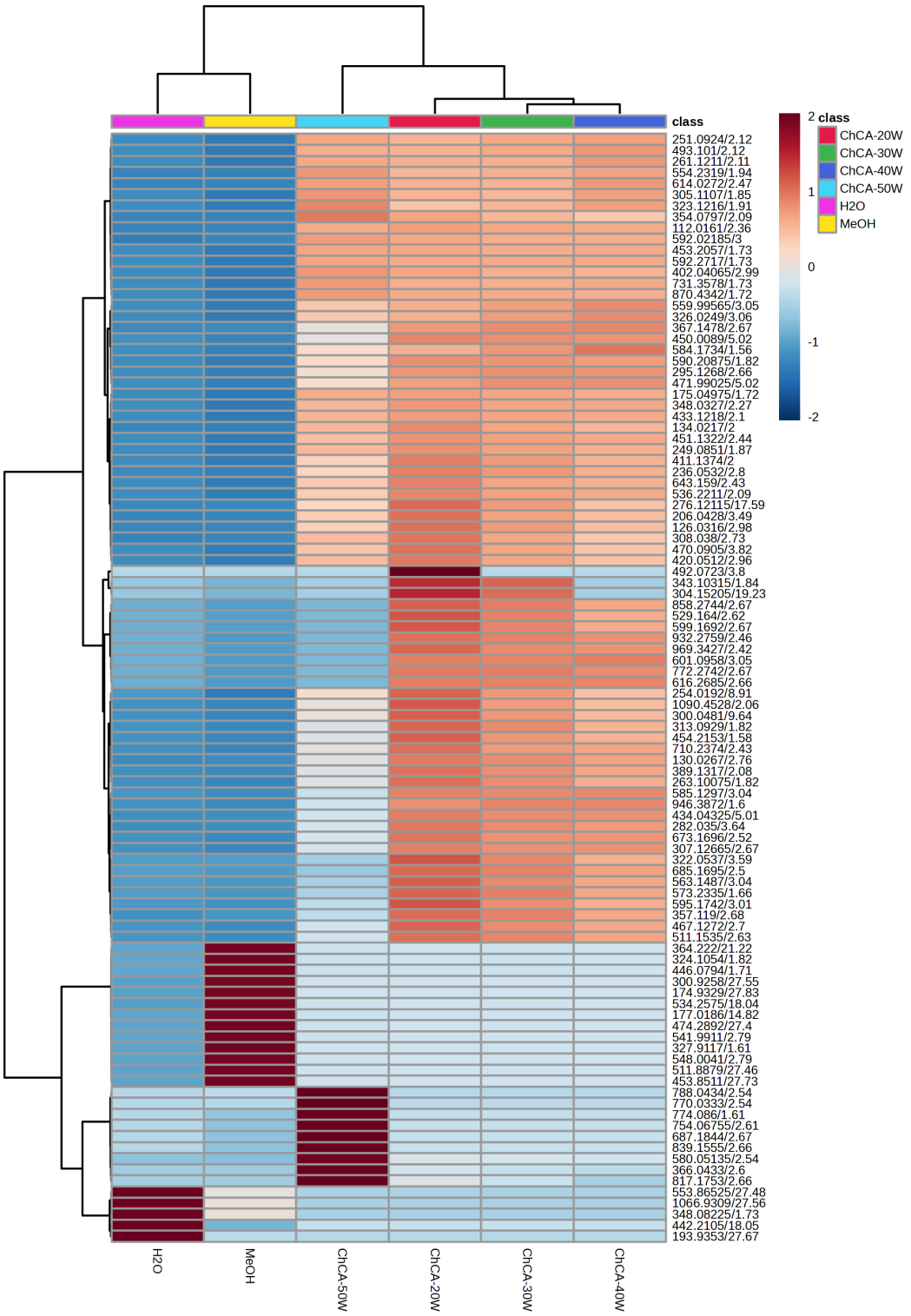

Fig. 3 The heat map according to the peak list analyzed in negative mode showing up to 100 compounds of the DCCH crude extract extracted using different solvents: ChCA-20W (red row), ChCA-30W (green row), ChCA-40W (navy blue row), ChCA-50W (light blue row), Milli-Q water (pink row), and methanol (yellow row).

ponents accounted for 94.7%, 4%, and 0.4% of the total variance, respectively. It seems that the group of compounds found in crude samples obtained from using ChCA-50W, water, and methanol as extractants shows a significant difference from others. This result is due to the effect of some compound masses on the clustering pattern. The compound masses (g/mole) that had an impact on the clustering from the first principal component to the second component were 112.0161, 130.0267, 174.0166, 192.0635, 313.0929, 206.0428, and 366.0433. Additionally, the compound masses (g/mole) for the first principal components to the third components that had an impact on clustering were 112.0161, 130.0267, 313.0929, 831.3481, 263.10075, 174.0166, 366.0433, and 392.0402. The clustering of the compounds extracted by water and methanol seems to be too close to each other. The positions of these 2 groups of substances should be separated completely because the number of compounds extractable by MeOH is as high as 1423 ± 246 compounds, while that of water can be extracted in the range of 407 ± 11 compounds. This is because there are not many compound masses that can influence the separation of those groups on the first component, and the separation of these 2 groups results in the third component having low loading values. It indicates that considerably different extractant solvents were used, which resulted in a different group and content of chemicals. This information can be confirmed by a heat map analysis (Fig. 3). A heat map analysis using the Ward clustering method and the Euclidean distance measurement found that it was possible to divide the extracted compounds limiting 100 compounds into 3 groups: those obtained from ChCA with water mixing (20–50%) and from Milli-Q water and methanol. The number of compounds influencing clustering in the crude sample obtained from ChCA with a water content of 20–40% as extractants is very similar, even though the concentrations vary. In addition, the amount of compound and concentration found in the crude sample obtained from using ChCA-50W, water, and methanol are significantly different. The results of this study demonstrated that several components found in crude samples obtained from using ChCA with various water contents as extractants differ from those obtained from methanol and water extractions. It could be supported by the report mentioned by Shi et al [48] showing that some lipophilic components that are insoluble in polar solvents like ethanol might be extracted using a DES solution. Enhancing hydrogen bonding could be improved in this solvent system. This was made possible by the highly delocalized conjugated system as well as the electron-donating and -absorbing groups. The solvent system became more ionic liquid-like due to the solvent's hyperpolarizability, which also increased hydrogen bonding between the refractory chemical components and the DES system. Additionally, with ChCA-50W extraction, we discovered a set of compounds that were quite similar to and different from those obtained by utilizing ChCA with a water concentration of 20–40%. As mentioned previously, the polarizability of DESs increased with the addition of water, and more water can make flavonoid extraction less effective [42, 43]. The extraction of bioactive substances is limited by the increased polarity of solvents. Furthermore, the decrease in compound extraction yields due to the significant dilution of DESs with water was explained as a decrease in hydrogen bond interaction between DES components and bioactive chemicals [44].

CONCLUSION

The DES prepared from ChCl and CA with a 1:2 mole ratio mixed with different water contents can be an alternative green solvent for extracting bioactive compounds in DCCH because their crude extract shows a high value of chemical content and antioxidant activity. The occurrence of hydrogen bonding between the chlorine anion of ChCl and the hydroxyl group on CA was indicated by FTIR. The strength of hydrogen bonding occurring in the medium tends to play a vital role in extraction efficiency. The addition of water to the DES reduces the viscosity and leads to better extraction efficiency. The levels of TPC, TFC, and AA were found to be highest in DES with 40% w/w additional water. Statistical data analysis using one-way ANOVA, PCA, and heat map plots could show that some components in crude samples extracted with DES significantly differed from those extracted with methanol and water. The results suggested that our green and sustainable extraction method could be practical for the extraction of polyphenols from agricultural wastes.

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