https://doi.org/10.17221/33/2026-CJFS

Antioxidant potential and antimicrobial activity of coloured onion peels extracted using ultrasound-assisted deep eutectic solvents

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Citation: Demir Ö., Baştürk F.N., Gerçek Y., Kara E.M., Kirbaşlar Ş.İ. (2026): Antioxidant potential and antimicrobial activity of coloured onion peels extracted using ultrasound-assisted deep eutectic solvents. Czech J. Food Sci., 44: 353–367.

Abstract: In this study, the most effective deep eutectic solvent (DES) for extracting antioxidants from purple onion peel was identified using total antioxidant capacity (TAC) values obtained by the CUPRAC assay. The DES systems used in this study consisted of choline chloride (ChCl) combined with ethylene glycol (EG), diethylene glycol (DiEG), triethylene glycol (TriEG), tetraethylene glycol (TetEG), 1,4-butanediol (1,4-but), and 1,2-propanediol (1,2-pro) at a molar ratio of 1 : 2 with 30% water content. All DESs were characterised in terms of Fourier transform infrared (FTIR) spectra, viscosity, density, and pH. Ultrasound-assisted extraction was optimised using response surface methodology, resulting in a maximum TAC of 582.96 mg Trolox equivalent (TE)·g^{-1} dry sample (DS) for ChCl : 1,4-but : H_2O, which was almost twice the amount achieved with 70% ethanol. Antioxidant parameters, including TAC, total phenolic content, total flavonoid content, and radical scavenging activities [2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)], were evaluated for purple, red, brown, and white onion peels. Antimicrobial activity was assessed using the broth microdilution method. HPLC analysis was employed to quantify phenolic and flavonoid compounds, revealing significant compositional differences among the onion peels. Overall, the results demonstrate that purple and red onion peels exhibit superior antioxidant and antimicrobial properties, supporting their potential as sustainable sources of natural antioxidants.

Keywords: ultrasound-assisted extraction; CUPRAC assay; phenolic compounds; flavonoids; HPLC analysis; *Allium cepa*

Supported by the Istanbul University–Cerrahpaşa (FDK-2024-37471) and the Scientific and Technological Research Council of Türkiye (TÜBİTAK; 1002-A Project No. 125M724). Additionally, one author received financial support from Tübitak under the 2211-A National PhD Scholarship Program.

https://doi.org/10.17221/33/2026-CJFS

Oxidative stress occurs when the production of reactive oxygen and nitrogen species exceeds cellular antioxidant capacity. Elevated levels of these species can damage essential biomolecules such as nucleic acids, proteins, and lipids, contributing to diseases including cancer, cardiovascular disorders, and neurodegenerative conditions. Natural antioxidants mitigate oxidative stress by neutralising free radicals and inhibiting chain reactions; therefore, their application is important for regulating oxidative damage (Warraich et al. 2020). Given the growing interest in natural antioxidant sources, agricultural by-products have attracted considerable attention as sustainable raw materials for bioactive compound recovery.

Onion (*Allium cepa* L.) is one of the most economically important horticultural crops worldwide, ranking second after tomato in global production. Its high adaptability to different climatic conditions and water availability allows cultivation in many regions. The onion processing industry generates more than 550 000 tonnes of waste annually, mainly onion peels. These peels contain high levels of bioactive compounds, particularly polyphenols and flavonoids, often exceeding those in the edible parts (Sagar et al. 2022). Consequently, onion peel waste has considerable potential for food industry applications and functional food development through appropriate extraction techniques (Kumar et al. 2022a). Plant materials are considered conventional sources of bioactive phytochemicals; however, their phytochemical composition may vary significantly among plant species and cultivars (Shiponi and Bernstein 2021), and can be strongly influenced by cultivation and environmental conditions (Song et al. 2023).

Extraction methods are generally classified as traditional or modern. Conventional techniques such as maceration, Soxhlet, and solvent extraction are often time-consuming, require large solvent volumes, and may cause thermal degradation of antioxidant compounds, reducing extraction efficiency. In addition, the use of hazardous and flammable solvents raises concerns regarding safety and waste management. Therefore, green and sustainable extraction methods have gained increasing attention for the efficient recovery of natural antioxidants.

Among modern green extraction approaches, ultrasound-assisted extraction has gained considerable attention due to its operational advantages and high extraction efficiency. Ultrasound-assisted extraction (UAE) is an efficient technique that shortens extraction time and reduces solvent consumption. It operates through ultrasonic cavitation, where pressure fluctuations generate bubbles that rapidly collapse, producing shockwaves and localised heat. This process disrupts plant cell walls, enhancing the release of bioactive compounds and improving solvent diffusion and analyte solubility. As a result, UAE increases phytochemical extraction efficiency and is widely applied in the food and pharmaceutical industries. However, solvent selection remains critical for extraction performance. Conventional solvents such as methanol, acetone, and ethanol may pose environmental and health risks due to their volatility, toxicity, and limited renewability. Therefore, environmentally friendly solvent systems are increasingly preferred (Rashid et al. 2022).

As a result, deep eutectic solvents (DES)-based systems have recently emerged as promising green solvent alternatives for the extraction of plant-derived bioactive compounds. DES are considered sustainable alternatives. In DES-based extraction, solvent removal is often unnecessary, allowing direct use of polyphenol-rich extracts in food, pharmaceutical, or cosmetic products. Moreover, DES can be prepared easily through a simple heating and mixing process. DES are eutectic mixtures formed by hydrogen bond acceptors (HBAs), such as quaternary ammonium or phosphonium salts, and hydrogen bond donors (HBDs), including amines, alcohols, amides, or carboxylic acids. Interactions between these components significantly lower the melting point of the mixture compared with that of the individual compounds. Among them, choline chloride (ChCl)-based DES are particularly promising due to their low cost, low toxicity, biodegradability, and strong hydrogen-bond interactions that enhance extraction efficiency and compound stability. ChCl can also be combined with various HBDs, providing flexibility in DES design (Wang et al. 2019).

Therefore, integrating DES systems with UAE may provide a synergistic approach for enhancing extraction performance while maintaining environmental sustainability. Combining UAE with DES offers a greener and more efficient extraction strategy. The low vapour pressure of DES enhances cavitation intensity, improving mass transfer and extraction yield. Previous studies report that ultrasound assisted deep eutectic solvent extraction systems provide higher total phenolic content (TPC) and radical scavenging activity than conventional solvents such as water and aqueous ethanol, indicating a synergistic effect between UAE and DES (Fu et al. 2021).

Based on these considerations, the present study was designed to investigate the extraction efficiency and bioactive potential of onion peel varieties using DES–

https://doi.org/10.17221/33/2026-CJFS

UAE systems. This study investigates the antioxidant potential of four onion peel varieties (purple, red, brown, and white) cultivated in Turkey, a major onion-exporting country. Six ChCl–alcohol-based DES were synthesised and characterised by Fourier transform infrared (FTIR) spectroscopy, viscosity, density, pH, and refractive index measurements. The most effective DES was selected based on the total antioxidant capacity (TAC) of purple onion peel extracts determined by the CUPRAC method.

Subsequently, UAE extraction parameters were optimised using the Box–Behnken experimental design. Under optimised conditions, antioxidant properties of the four onion peel types were evaluated through TAC, TPC, total flavonoid content (TFC), and radical scavenging activities [2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)]. Antimicrobial activities were also assessed, and phenolic and flavonoid profiles were identified and quantified using HPLC (high-performance liquid chromatography).

Overall, this study aims to support agricultural waste valorisation by demonstrating the effectiveness of DES–UAE systems for extracting antioxidant compounds and by comparatively evaluating the antioxidant, antimicrobial, and phenolic profiles of different onion peel varieties, an area for which limited information is currently available in the literature.

MATERIAL AND METHODS

Material. All chemicals used in this study were obtained from Sigma-Aldrich (Germany). For DES synthesis, ChCl, ethylene glycol (EG), diethylene glycol (DiEG), triethylene glycol (TriEG), tetraethylene glycol (TetEG), 1,4-butanediol (1,4-but), and 1,2-propanediol (1,2-pro) were used.

The CUPRAC assay employed copper(II) chloride, neocuproine, ammonium acetate, and Trolox. DPPH radical scavenging activity was determined using DPPH, methanol, and Trolox, while the ABTS assay utilised ABTS, potassium persulfate ($K_2S_2O_8$), ethanol, and Trolox. TPC was measured by the Folin–Ciocalteu method using Folin–Ciocalteu reagent, sodium carbonate (Na_2CO_3), ethanol, and gallic acid.

TFC was determined using aluminium chloride hexahydrate, sodium nitrite, sodium hydroxide, ethanol, and quercetin. HPLC analyses were performed with formic acid and methanol as mobile phase components, and kaempferol, quercetin, myricetin, ferulic acid, *p*-coumaric acid, syringic acid, rutin, and caffeic acid as analytical standards.

DES preparation and characterisation. In DES synthesis, ChCl served as HBA, while polyols including EG, DiEG, TriEG, TetEG, 1,4-but, and 1,2-pro acted as HBDs. ChCl and the selected HBD were accurately weighed (± 0.001 g), mixed with distilled water (30% *v/v*), and stirred at 333.15 K using a heated magnetic stirrer until a homogeneous liquid was obtained (Bener et al. 2022).

The addition of water decreases DES viscosity, improving mass transfer and diffusion during extraction. However, excessive water may weaken DES–phenolic interactions due to the limited solubility of phenolic compounds in water. Therefore, water content was limited to 30% (*v/v*) to balance viscosity reduction and extraction efficiency (Ozturk et al. 2018). The compositions and abbreviations of the synthesized DES are listed in Table 1.

DES density was measured at 25 °C using an Anton Paar density meter (Austria). pH values were determined with a digital pH meter (Mettler Toledo, SevenMulti), and viscosity was measured using a rotational rheometer (Discovery Hybrid Rheometer-1) at 25 °C. FTIR spectra were recorded with KBr disks (sample/KBr = 1/200) using a Cary 630 spectrophotometer (Agilent, USA) to identify functional groups and confirm the DES structure.

Table 1. The abbreviations and components of deep eutectic solvents

Abbreviations	HBA	HBD	Molar ratio(HBA : HBD)	Water content (% *w/w*)
ChCl : EG : H_2O	ChCl	EG	1 : 2	30
ChCl : DiEG : H_2O	ChCl	DiEG	1 : 2	30
ChCl : TriEG : Hz_2O	ChCl	TriEG	1 : 2	30
ChCl : TetEG : H_2O	ChCl	TetEG	1 : 2	30
ChCl : 1,4-but : H_2O	ChCl	1,4-but	1 : 2	30
ChCl : 1,2-pro : H_2O	ChCl	1,2-pro	1 : 2	30

HBA – hydrogen bond acceptor; HBD –hydrogen bond donor; ChCl – choline chloride; EG – ethylene glycol; DiEG – diethylene glycol; TriEG – triethylene glycol; TetEG – tetraethylene glycol; 1,4-but – 1,4-butanediol; 1,2-pro –1,2-propanediol

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Plant materials. Purple onion (*Allium cepa* L. var. purple onion) samples were obtained from the Erdek district of Balıkesir Province, red onion (*Allium cepa* L. var. red onion) samples from Çorum Province, brown onion (*Allium cepa* L. var. cepa) samples from the Karacabey district of Bursa Province, and white onion (*Allium cepa* L. var. alba) samples from Erdek (Balıkesir). All onion varieties used in this study possessed geographical indication certification.

Onion peels were cleaned to remove damaged or infected parts, then dried in an oven (Electromag M3025P) at 75 °C for 8 h. The dried peels were ground using a household grinder and sieved to obtain a particle size of approximately 500 μm. The powdered samples were stored in vacuum-sealed plastic containers until analysis.

UAE procedure. Onion peel extraction was performed using an ultrasonic processor (VCX 750, USA) equipped with a probe to transmit acoustic energy into the extraction medium. For each experiment, 0.5 g of onion peel sample was used. All extractions and analytical measurements were performed in triplicate.

Ultrasound was applied at a fixed frequency of 20 kHz and 75% amplitude (Bener 2019), with a pulse mode of 5 s on and 2 s off. The applied power ranged from 500 to 700 W, extraction time from 10 to 30 min, and the liquid-to-solid ratio ($mL \cdot g^{-1}$) from 60 to 100.

After extraction, the mixtures were centrifuged (Nüve NF 200) at 5 000 rpm for 10 min. The supernatants were filtered through a 0.45 μm syringe filter and stored at 4 °C until analysis. The experimental workflow is presented in Figure 1.

TAC assay. TAC of onion peel extracts was determined using the CUPRAC method (Bener et al. 2022), which is based on the reduction of the Cu(II)–neocuproine complex to Cu(I)–neocuproine in the presence of antioxidant compounds, producing a yellow–orange complex measured at 450 nm. Briefly, 1 mL of 10 mM Cu(II), 1 mL of 7.5 mM neocuproine, and 1 mL of 1 M ammonium acetate buffer were mixed with the sample extract and distilled water to obtain a final volume of 4.1 mL. After incubation for 30 min at room temperature, absorbance was measured at 450 nm against a reagent blank. TAC values were expressed as Trolox equivalents [mg $TE \cdot g^{-1}$ dry sample (DS)] using a calibration curve prepared with Trolox standards.

TPC assay. TPC of the extracts was determined using the Folin–Ciocalteu method with gallic acid as the reference standard (Magalhães et al. 2010). Briefly, 50 μL of sample extract was mixed with 50 μL of Folin–Ciocalteu reagent diluted 1: 5 (*v/v*) with distilled water, followed by the addition of 100 μL of 0.35 M sodium hydroxide. After incubation for 30 min at room temperature, the absorbance of the blue complex was measured at 760 nm. TPC values were expressed as gallic acid equivalents (mg $GAE \cdot g^{-1}$ DS) using a calibration curve prepared with gallic acid standards (Mayda et al. 2020).

TFC assay. TFC of the extracts was determined using a modified aluminium chloride colorimetric method based on Zhishen et al. (1999). Briefly, 1 mL of extract was mixed with 0.3 mL of 10% (*w/v*) aluminium chloride hexahydrate and 0.3 mL of 5% (*w/v*) sodium nitrite solution, followed by the addition of 2 mL of 1 M sodium hydroxide and 2.4 mL of distilled water. After thorough mixing, the absorbance was measured at 510 nm against a reagent blank. TFC values were expressed as quercetin equivalents (mg $QE \cdot g^{-1}$ DS) using a calibration curve prepared with quercetin standards (Mayda et al. 2020).

ABTS assay. ABTS radical scavenging activity of onion peel extracts was determined using the ABTS assay. The ABTS+ radical was generated by dissolving ABTS (7.0 mM) in 50 mL of distilled water followed by the addition of potassium persulfate (2.45 mM) and incubation in the dark at room temperature for 12–16 h. For analysis, the sample extract was mixed with methanol and 1 mL of ABTS+ radical working solution diluted 1: 10 (*v/v*) with ethanol, and the absorbance was measured at 734 nm exactly 6 min after reagent addition against an ethanol blank. The ABTS radical scavenging capacity was expressed as Trolox equivalents (mg $TE \cdot g^{-1}$ DS) using a calibration curve prepared with Trolox standards (Bener et al. 2022).

DPPH assay. The DPPH free radical scavenging activity of onion peel extracts was evaluated using the DPPH assay. In this method, antioxidant compounds reduce the stable DPPH radical, causing a colour change from purple to yellow that is monitored spectrophotometrically at 515 nm. For the assay, the sample extract was mixed with methanol and 2 mL of 0.2 mM DPPH methanolic solution, and the reaction mixture was incubated in the dark at room temperature for 30 min. After incubation, absorbance was measured at 515 nm against a methanol blank. The DPPH scavenging capacity was expressed as Trolox equivalents (mg $TE \cdot g^{-1}$ DS) using a calibration curve prepared with Trolox standards (Altun et al. 2011).

HPLC analyses. Polyphenolic compounds were analysed using an Agilent 1260 Infinity series HPLC system equipped with a quaternary pump, autosampler, column oven, and diode array detector (DAD) using a C18 column (Agilent ZORBAX Eclipse Plus

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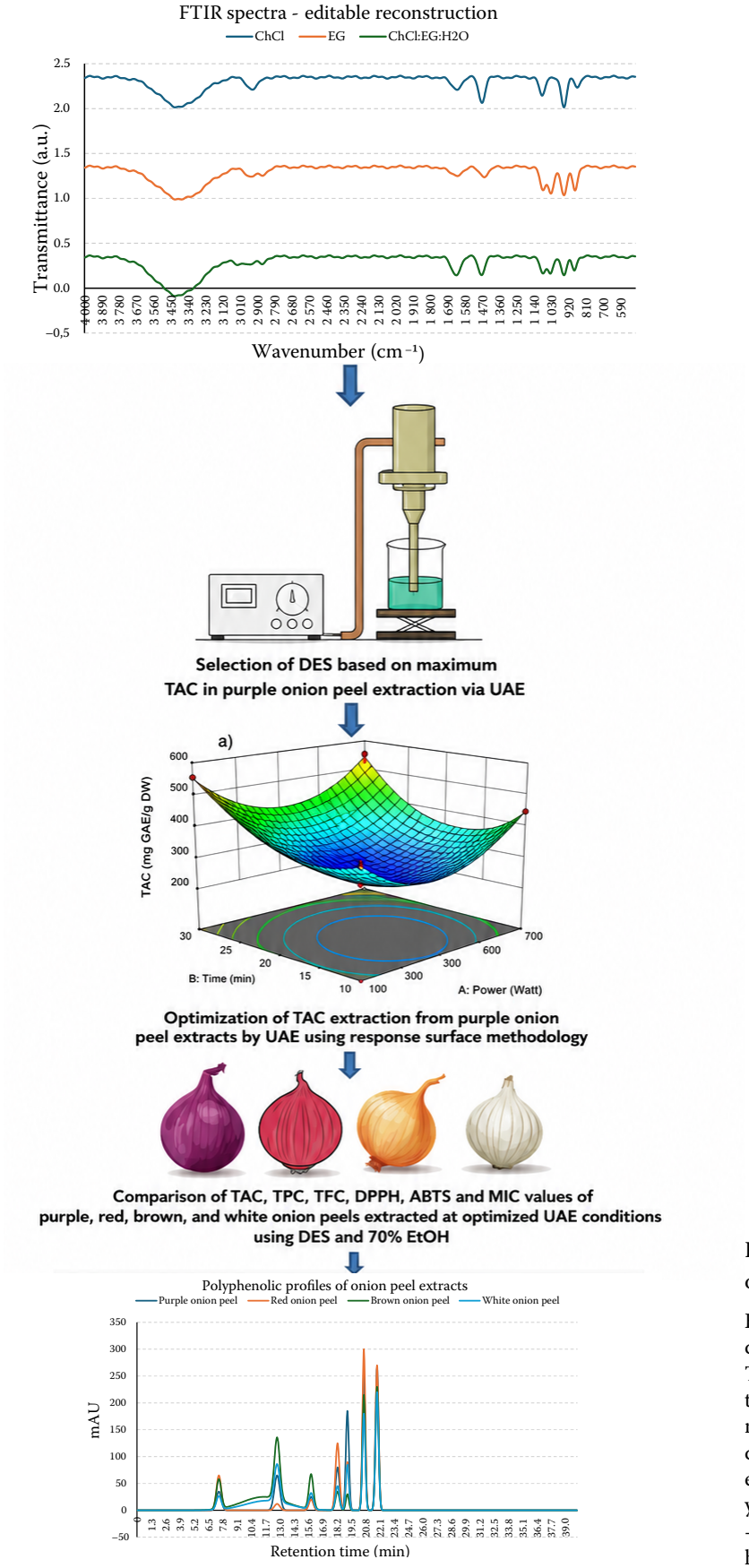

Figure 1. Experimental flowchart of thestudy

EG – ethylene glycol; ChCl – choline chloride; DES – deep eutectic solvents; TAC – total antioxidant capacity; TPC – total phenolic content; TFC – total flavonoid content; MIC – minimum inhibitory concentration; UAE – ultrasound assisted extraction; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH – 2,2-diphenyl-1-picrylhydrazyl; HPLC – high performance liquid chromatography

https://doi.org/10.17221/33/2026-CJFS

Table 2. Calibration parameters and regression equations used for the quantification of phenolic compounds

Compound name	Detection wavelengths (nm)	Retention time (min)	Calibration ranges (ppm)	Equation	R^2
Caffeic acid	310	21.50	5–100	$y = 609.66x + 4\,128.9$	0.98
Syringic acid	280	22.66	5–100	$y = 179.14x + 368.19$	0.99
p-coumaric acid	310	29.21	5–100	$y = 579.35x + 479.29$	0.99
Ferulic acid	310	33.63	5–100	$y = 417.64x + 567.26$	0.99
Rutin	254	35.07	5–100	$y = 139.35x + 3\,767.3$	0.97
Myricetin	254	37.62	5–100	$y = 322.57x + 7\,976.8$	0.99
Quercetin	254	38.94	5–100	$y = 452.03x + 8\,069.4$	0.99
Kaempferol	254	41.34	5–100	$y = 366.93x - 234.22$	0.99

C18, 4.6 × 250 mm, 5 µm particle size). The mobile phase consisted of 0.1% (*v/v*) formic acid in ultrapure water (solvent A) and 0.1% (*v/v*) formic acid in acetonitrile (solvent B). The gradient elution program was set as follows: 0–2 min, 5% B; 2–27 min, 15% B; 27–37 min, 50% B; 37–47 min, 95% B; 47–58 min, 95% B; 58–60 min, 5% B; and 60–70 min, 5% B. The flow rate was maintained at 0.2 mL·min^{-1} and the injection volume was 10 µL (Bayram et al. 2020).

Phenolic acids were identified and quantified using *p*-coumaric acid, syringic acid, ferulic acid, and caffeic acid as external standards, while rutin, myricetin, kaempferol, and quercetin were used as standards for flavonoids. Calibration curves were prepared for each standard over a concentration range of 5–100 ppm. Detection wavelengths were set at 254 nm for rutin, myricetin, quercetin, and kaempferol; 280 nm for syringic acid; and 310 nm for caffeic acid, *p*-coumaric acid, and ferulic acid.

Figure 2 illustrates the chromatographic peaks, retention times, and detection wavelengths of the identified phenolic compounds. The calibration parameters and regression equations of the quantified phenolics within the studied concentration range are presented in Table 2.

Statistical analyses. Experimental design and regression analysis were performed using Design-Expert software (version 13.0.15, Stat-Ease Inc., USA). The effects of UAE power (300–700 W), extraction time (10–30 min), and liquid-to-solid ratio (60–100 mL·g^{-1}) on TAC were optimised using a Box–Behnken response surface design. All experiments and analytical measurements were performed in triplicate, and the results were expressed as mean ± standard deviation. Statis-

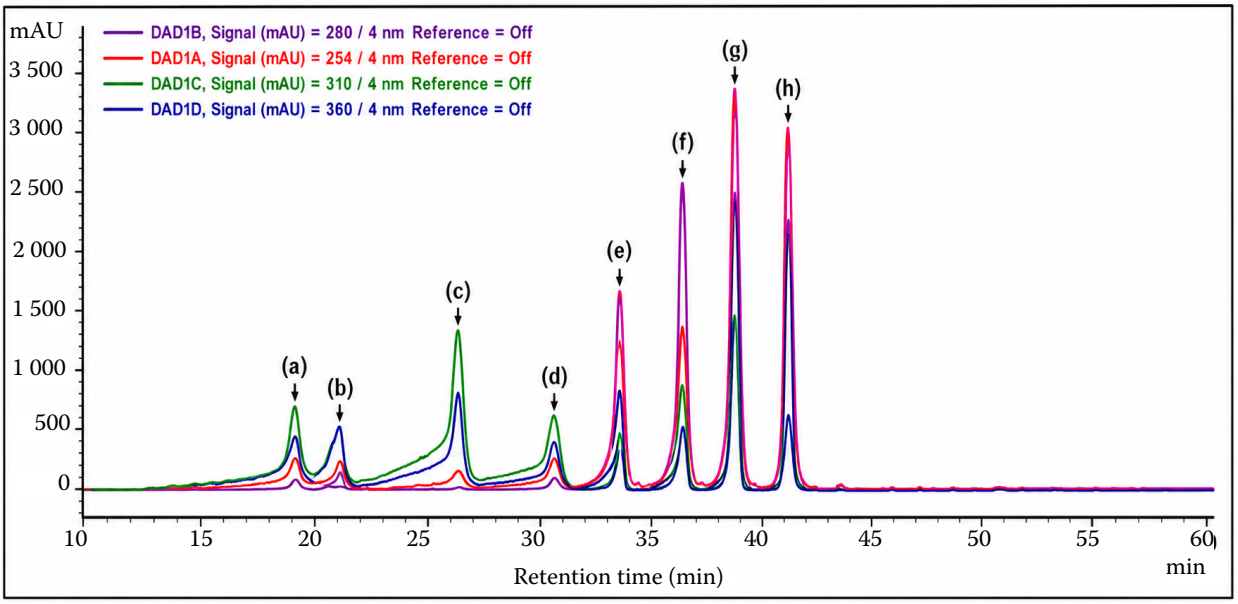

Figure 2. Chromatographic peaks, retention times, and detection wavelengths of the identified phenolic compounds: (a) caffeic acid, (b) syringic acid, (c) *p*-coumaric acid, (d) ferulic acid, (e) rutin, (f) myricetin, (g) quercetin, and (h) kaempferol

https://doi.org/10.17221/33/2026-CJFS

tical significance and model adequacy were evaluated by ANOVA at $P < 0.05$ based on the F-value and coefficient of determination (R^2) (Ozturk et al. 2018). The optimisation study and ANOVA analysis were conducted specifically for TAC as the primary response variable of the Box–Behnken design.

Evaluation of antimicrobial efficacy. The minimum inhibitory concentrations (MICs) of extracts were determined according to the broth microdilution guidelines of the Clinical and Laboratory Standards Institute (CLSI) (Pfaller et al. 2002; Subramaniam and Nandan 2011). Four Gram-negative bacteria – *Pseudomonas aeruginosa* ATCC 27853 (PA), *Klebsiella pneumoniae* ATCC 4352 (KP), *Proteus mirabilis* ATCC 14153 (PM), and *Escherichia coli* ATCC 25922 (EC); four Gram-positive bacteria – *Staphylococcus aureus* ATCC 29213 (SA), methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus epidermidis* ATCC 12228 (SE), and *Enterococcus faecalis* ATCC 29212 (EF); and three fungal strains – *Candida albicans* ATCC 10231 (CA), *Candida parapsilosis* ATCC 22019 (CP), and *Candida tropicalis* ATCC 750 (CT) – were used in this study.

Mueller–Hinton broth was employed for bacterial assays, while RPMI-1640 medium was used for yeast assays, in accordance with CLSI recommendations. Test compounds were dissolved in dimethyl sulfoxide (DMSO), and two-fold serial dilutions ranging from 1 250 to 0.06 μg·mL^{-1} were prepared in the corresponding media.

Inocula were prepared from 4–6 h bacterial cultures and 24 h yeast cultures, adjusted to a 0.5 McFarland standard, and subsequently diluted to final concentrations of 5×10^5 CFU·mL^{-1} for bacteria and 0.5×10^3–2.5×10^3 CFU·mL^{-1} for yeasts. Microplates were sealed to prevent evaporation and incubated at 35 °C for 18–20 h for bacteria (Mueller–Hinton broth) and 46–50 h for yeasts (RPMI-1640 medium).

The MIC was defined as the lowest concentration of extract that completely inhibited visible microbial growth. Assays were performed in triplicate and evaluated against appropriate controls. Standard antibiotics (ceftazidime, cefuroxime sodium, and amikacin) and antifungal agents (clotrimazole and amphotericin B) were used as reference compounds, and the obtained MIC values were within the CLSI-recommended ranges.

RESULTS AND DISCUSSION

DES characterisation. The physicochemical properties of the synthesised DES at 25 °C are summarised in Table 3. All DES were prepared using polyol-based HBDs containing two hydroxyl groups at a molar ratio of 1 : 2 (HBA : HBD) with 30% (*v/v*) water. The similar viscosity values observed among the systems can be attributed to the comparable structures of the HBDs and identical preparation conditions (Wan Mahmood et al. 2019).

For industrial applications, solvents with viscosities below 0.1 Pa·s are generally preferred to ensure efficient mass transfer, whereas sugar-based DES often exhibit much higher viscosities (≥ 5 Pa·s), limiting their extraction performance (Xu et al. 2019). The DES investigated in this study showed viscosities below 0.1 Pa·s, indicating their suitability for industrial extraction processes.

The densities of the synthesised DES were comparable to that of water, consistent with reports that DES density increases with the number of hydroxyl groups in the HBD structure (Jurić et al. 2021). Since all HBDs contained two hydroxyl groups, their densities were similar (Figure 3). Among the systems, ChCl: EG: H_2O exhibited the lowest viscosity, likely due to the shorter carbon chain of ethylene glycol. Refractive index values ranged from 1.4 to 1.5, and all DES showed near-neutral pH values, which may help minimise undesirable

Table 3. Physicochemical characteristics of the prepared deep eutectic solvents

Abbreviations	Viscosity (Pa.s)	Density (g·cm^{-3})	pH	Refractive index (n_D)
ChCl : EG : H_2O	0.020 2	1.084 01	4.422	1.424 37
ChCl : DiEG : H_2O	0.029 4	1.094 07	4.264	1.428 20
ChCl : TriEG : H_2O	0.024 8	1.100 93	3.257	1.432 82
ChCl : TetEG : H_2O	0.026 1	1.102 26	3.419	1.425 44
ChCl : 1,4-but : H_2O	0.026 7	1.052 01	4.361	1.429 96
ChCl : 1,2-pro : H_2O	0.035 4	1.066 84	5.657	1.428 51

ChCl – choline chloride; EG – ethylene glycol; DiEG – diethylene glycol; TriEG – triethylene glycol; TetEG – tetraethylene glycol; 1,4-but – 1,4-butanediol; 1,2-pro –1,2-propanediol

https://doi.org/10.17221/33/2026-CJFS

Table 4. Types of DES and corresponding TAC values

Types of DES	TAC (mg TE·g^{-1} DS)
ChCl : EG : H_2O	261.23
ChCl : DiEG : H_2O	262.93
ChCl : TriEG : H_2O	361.72
ChCl : TetEG : H_2O	247.13
ChCl : 1,4-but : H_2O	467.90
ChCl : 1,2-pro : H_2O	288.51

DES – deep eutectic solvent; TAC – total antioxidant capacity; TE – trolox equivalents; DS – dry sample; ChCl – choline chloride; EG – ethylene glycol; DiEG – diethylene glycol; TriEG – triethylene glycol; TetEG – tetraethylene glycol; 1,4-but – 1,4-butanediol; 1,2-pro –1,2-propanediol

acidic or basic effects during bioactive compound extraction.

FTIR analysis was performed to confirm DES formation by comparing the spectra of the individual components and synthesised systems (Figure 4). Significant shifts in characteristic absorption bands indicated changes in molecular interactions after DES formation. Broad O-H stretching bands observed at 3 200–3 550 cm^{-1} confirmed the presence of hydroxyl groups and extensive hydrogen bonding within the DES structure. N-H stretching vibrations were detected at 3 015–3 026 cm^{-1}, while C-H stretching bands appeared at 2 800–3 000 cm^{-1}. Additional bands corresponding to N-H bending (1 640–1 651 cm^{-1}), C-H bending (1 476–1 479 cm^{-1}), and C-N stretching around 954 cm^{-1} further supported the structural interactions between DES components. Among the hydrogen bond donors, EG exhibited lower O-H stretching frequencies than DiEG, TriEG, and TetEG, indicating stronger hydrogen bonding interactions with ChCl. Similar

Table 5. Levels of independent variables optimised in the Box–Behnken design

Factor	Variable	Level		
		–1	0	1
A	power (Watt)	300	500	700
B	time (min)	10	20	30
C	L/S ratio	60	80	100

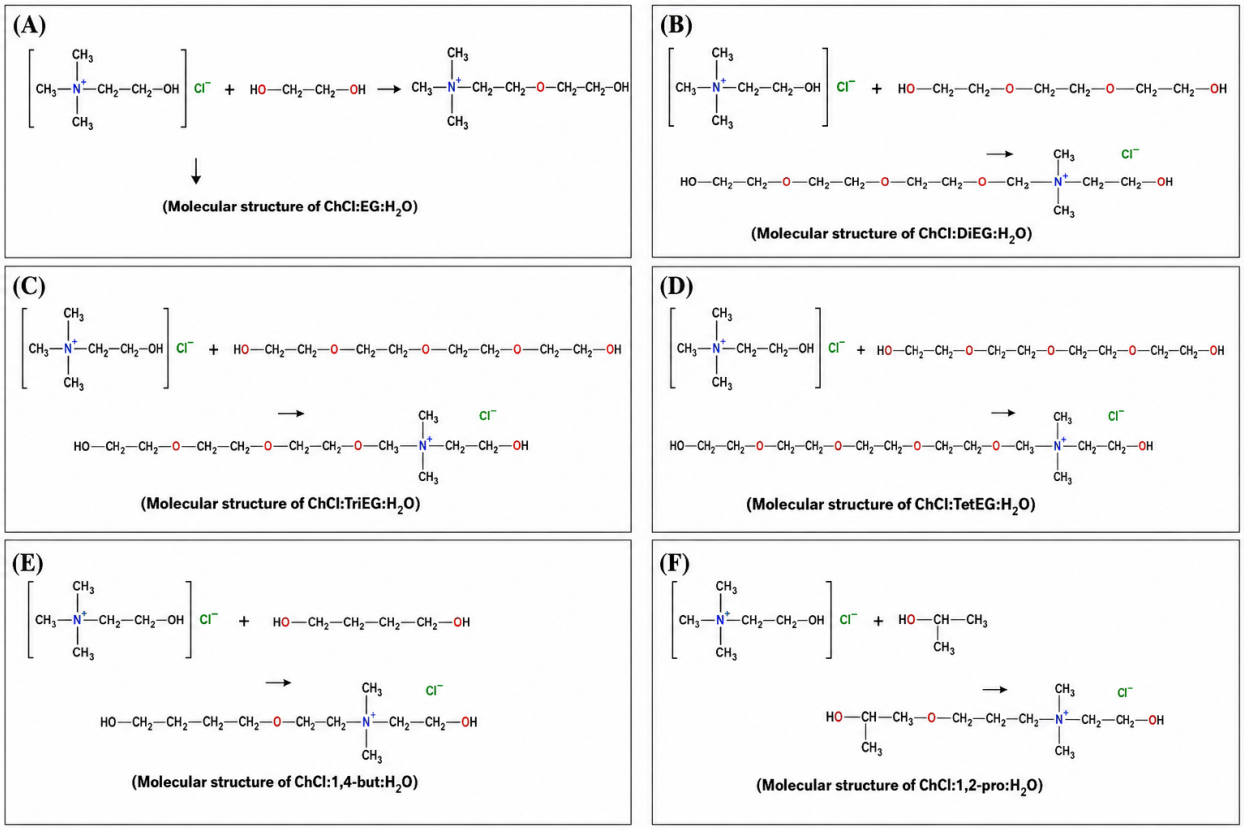

Figure 3. Proposed molecular structures and hydrogen-bonding interactions of ChCl-based DES: (A) ChCl: EG: H_2O; (B) ChCl: DiEG: H_2O; (C) ChCl: TriEG: H_2O; (D) ChCl: TetEG: H_2O; (E) ChCl: 1,4-but: H_2O; and (F) ChCl: 1,2-pro: H_2O

ChCl – choline chloride; EG – ethylene glycol; DiEG – diethylene glycol; TriEG – triethylene glycol; TetEG – tetraethylene glycol; 1,4-but – 1,4-butanediol; 1,2-pro –1,2-propanediol

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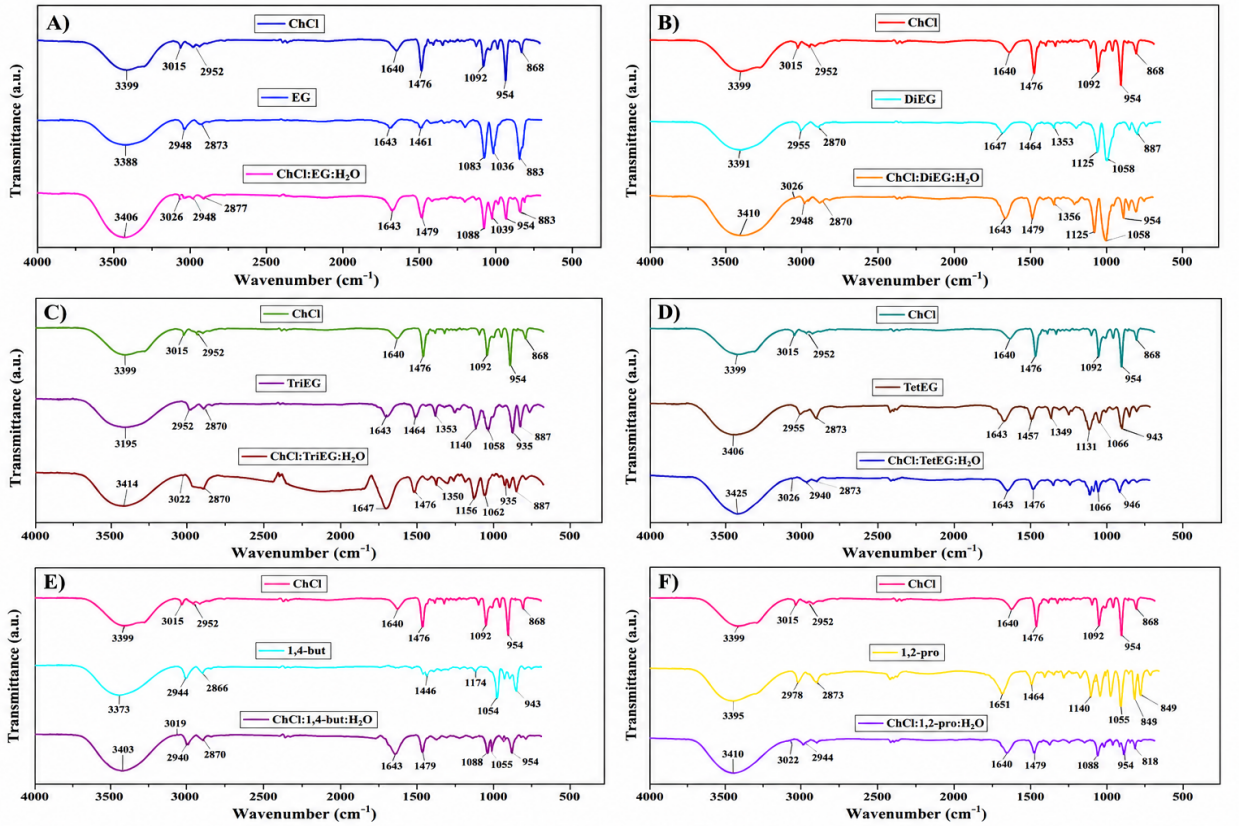

Figure 4. Fourier transform infrared (FTIR) spectra of ChCl-based deep eutectic solvents (DES) components and corresponding DES systems

(A) ChCl, EG, and ChCl: EG: H2O DES; (B) ChCl, DiEG, and ChCl: DiEG: H2O DES; (C) ChCl, TriEG, and ChCl: TriEG: H2O DES; (D) ChCl, TetEG, and ChCl: TetEG: H2O DES; (E) ChCl, 1,4-but, and ChCl: 1,4-but: H2O DES; (F) ChCl, 1,2-pro, and ChCl: 1,2-pro: H2O DES; ChCl – choline chloride; EG – ethylene glycol; DiEG – diethylene glycol; TriEG – triethylene glycol; TetEG – tetraethylene glycol; 1,4-but – 1,4-butanediol; 1,2-pro –1,2-propanediol

shifts in N-H and C-H vibrations after DES formation confirmed the establishment of intermolecular hydrogen bonds within the eutectic systems (Ibrahim et al. 2019; Majid et al. 2019; Doldolova et al. 2021; Jafari et al. 2022; Rashid et al. 2022). These spectral shifts and broadenings not only confirmed DES formation but also suggested that the strength of hydrogen-bond interactions may influence the physicochemical properties of the DES systems, such as viscosity, polarity, and extraction performance. Therefore, FTIR analysis provided important insights into the intermolecular interactions governing the efficiency of the DES-based extraction systems.

DES selection. DES can form extensive hydrogen-bonding networks and act as both HBDs and HBAs, enabling effective interactions with plant bioactive compounds. Polyol-based DES are particularly efficient for phenolic extraction due to strong interactions between phenolic hydroxyl groups and the –OH and/or Cl^- groups of DES components (Alsaud et al. 2021).

To identify the most effective solvent, purple onion peel extracts were used as the model system. UAE was performed under fixed conditions (500 W, 30 min, L/S ratio 100 mL·g^{-1}, 50 °C, 75% amplitude). TAC values obtained with different DES are presented in Table 4. The highest TAC (467.29 mg TE·g^{-1} DS) was obtained using ChCl : 1,4-but : H_2O, whereas the lowest value (288.51 mg TE·g^{-1} DS) was observed for ChCl : 1,2-pro : H_2O.

The extraction efficiency of ChCl-based DES is strongly influenced by the number, position, and chain length of hydroxyl groups in HBDs. In 1,2-pro, the close proximity of hydroxyl groups creates steric hindrance, limiting solvent–solute interactions and resulting in lower TAC values. In contrast, DES containing longer alkyl chains with terminal hydroxyl groups may reduce steric effects and promote stronger interactions with phenolic compounds, leading to higher extraction efficiency (Wan Mahmood et al. 2019).

https://doi.org/10.17221/33/2026-CJFS

Table 6. ANOVA analysis of the quadratic model

Source	Sum of Squares	df	Mean Square	*F*-value	*P*-value
Model	1.469E+05	9	16 320.68	46.33	< 0.000 1
A – power	32.40	1	32.40	0.0920	0.770 5
B – time	23 318.28	1	23 318.28	66.20	< 0.000 1
C – L/S ratio	16 526.53	1	16 526.53	46.92	0.000 2
AB	1 431.87	1	1 431.87	4.07	0.083 6
AC	7 635.26	1	7 635.26	21.68	0.002 3
BC	707.29	1	707.29	2.01	0.199 4
A^2	48 720.15	1	48 720.15	138.31	< 0.000 1
B^2	35 507.28	1	35 507.28	100.80	< 0.000 1
C^2	4 700.26	1	4 700.26	13.34	0.008 1
Residual	2 465.70	7	352.24	–	–
Lack of fit	2 115.70	3	705.23	8.06	0.026
Pure error	350.00	4	87.50	–	–
Corrected total	1.494E+05	16			

Based on these results, ChCl : 1,4-but : H_2O was selected as the optimal solvent for further experiments, and the effects of UAE parameters (extraction time, power, and L/S ratio) on TAC were subsequently optimised using this system.

TAC optimisation. During optimisation, temperature was fixed at 50 °C to balance reduced DES viscosity and improved mass transfer while preventing thermal degradation of bioactive compounds and loss of cavitation efficiency. A duty cycle of 71% (5 s on, 2 s off) was maintained throughout the UAE process to enhance extraction efficiency while avoiding excessive heat generation and degradation of thermolabile compounds (Rashid et al. 2022).

Optimisation experiments were conducted using purple onion peels with ChCl : 1,4-but : H_2O as the solvent. The effects of ultrasound power (A), extraction time (B), and liquid-to-solid ratio (C) on TAC were evaluated using a Box–Behnken design (Table 5). The experimental data were fitted to a second-order polynomial model describing the relationship between TAC and the independent variables.

Model adequacy was confirmed by ANOVA (Table 6). The model was highly significant (F = 46.33, P < 0.000 1), indicating a strong relationship between the variables and TAC. Among the parameters, extraction time (B), L/S ratio (C), the interaction term AC, and all quadratic terms (A^2, B^2, C^2) significantly influenced TAC at the 95% confidence level.

Model fit statistics (Table 7) further confirmed the reliability of the regression model. The close agreement between R^2 and adjusted R^2 indicated that the selected variables adequately explained the variation in TAC. Additionally, the coefficient of variation (4.93%) demonstrated high experimental precision and data consistency.

The combined effects of the extraction parameters are illustrated in the response surface plots (Figure 5). Increasing ultrasound power and extraction time enhanced TAC by intensifying cavitation and promoting cell wall disruption, thereby facilitating the release of antioxidant compounds. However, excessively high power levels may cause degradation of phenolic compounds; therefore, the maximum power was limited to 700 W (Fu et al. 2021). The liquid-to-solid ratio also significantly influenced TAC. Higher solvent volumes improved solvent–solute interactions and enhanced compound diffusion, leading to higher TAC values. In contrast, lower L/S ratios limited extraction efficiency due to insufficient solvent availability for hydrogen-bond interactions. Similarly, longer extraction times combined with higher L/S ratios increased TAC

Table 7. ANOVA model fit statistics

Statistic	Value
SD	18.77
Mean	380.49
CV %	4.93
R^2	0.983 5
Adjusted R^2	0.962 3
Adequate precision	19.057 4

https://doi.org/10.17221/33/2026-CJFS

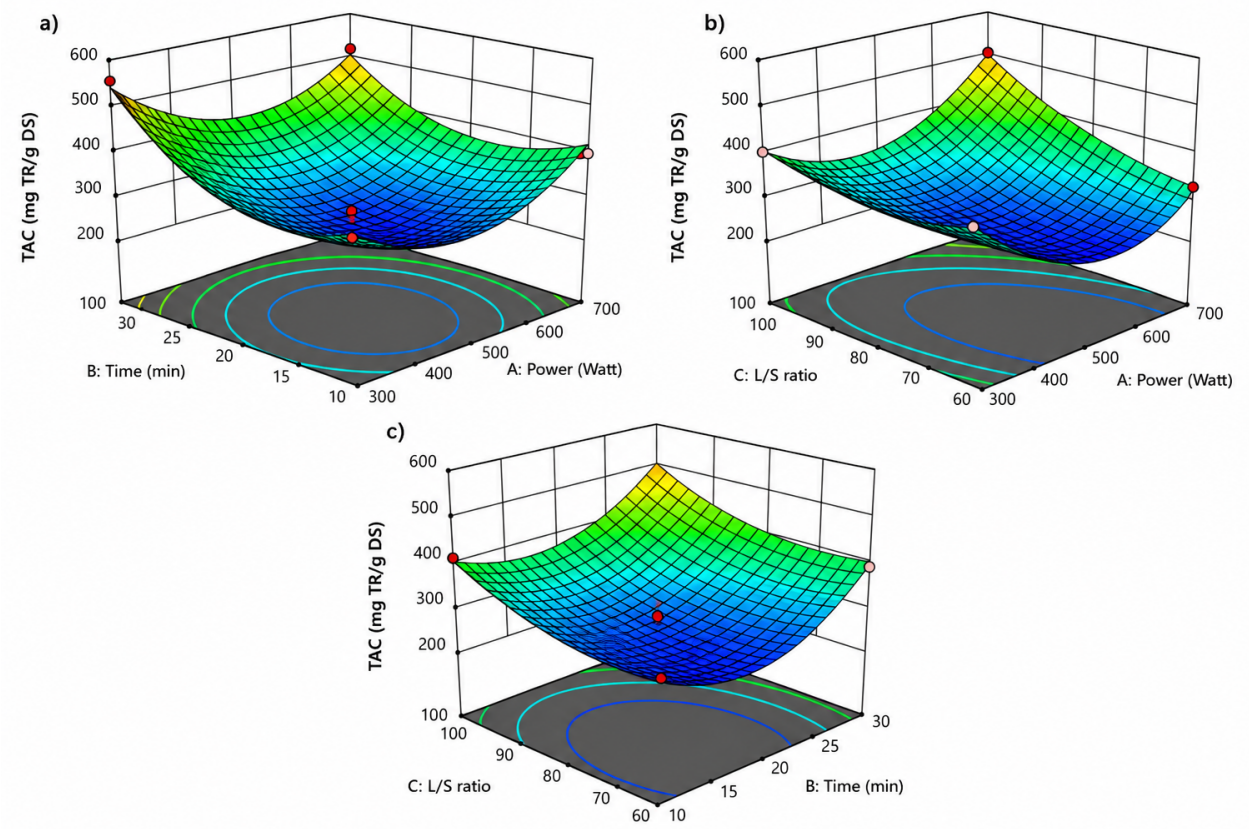

Figure 5. 3D response plots of the variables: (A) time vs power; (B) L/S ratio vs power; and (C) L/S ratio vs time

TAC – total antioxidant capacity; TE – trolox equivalents; DS – dry sample

by promoting greater diffusion and dissolution of antioxidant compounds. Under optimal conditions predicted by the model (298 W, 30 min, L/S ratio 100), the predicted TAC was 598.46 mg TE·g^{-1} DS. Experimental validation yielded a TAC of 582.96 mg TE·g^{-1} DS, with only 2.66% deviation, confirming the high predictive accuracy of the model.

Antioxidant activity and HPLC analysis at optimised UAE parameters. The results of UAE showed that the selected DES produced higher TAC values for purple, red, and white onion peel samples compared with extraction using 70% EtOH (Table 8). For example, the TAC of purple onion peel extract obtained with DES (582.95 mg TE·g^{-1} DS) was nearly twice that obtained with EtOH (311.18 mg TE·g^{-1} DS), indicating the superior extraction efficiency of DES and its potential as an environmentally friendly alternative solvent.

Table 8. Antioxidant activity levels in onion peel extracts according to solvent type under optimised ultrasound assisted extraction (UAE) conditions (298 W ultrasonic power, 30 min extraction time, and L/S ratio of 100 mL·g^{-1})

Peel	TAC (mg TE·g^{-1} DS)		DPPH (mg TE·g^{-1} DS)		ABTS (mg TE·g^{-1} DS)		TPC (mg GAE·g^{-1} DS)		TFC (mg QE·g^{-1} DS)	
	DES	%70 EtOH	DES	%70 EtOH	DES	%70 EtOH	DES	%70 EtOH	DES	%70 EtOH
Purple onion	582.95	311.18	101.32	106.97	137.26	128.30	234.43	286.78	259.14	247.18
Red onion	208.28	177.73	64.66	51.91	86.33	44.91	126.77	147.87	100.44	127.75
Brown onion	127.46	192.70	54.49	49.32	53.84	38.18	99.67	110.50	96.61	115.49
White onion	12.30	9.79	5.75	6.79	7.86	5.77	8.07	7.37	8.29	7.86

DES – deep eutectic solvent; DS – dry sample; TAC – total antioxidant capacity; DPPH – 2,2-diphenyl-1-picrylhydrazyl; ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); TPC – total phenolic content; TFC – total flavonoid content; TE – trolox equivalents; GAE – gallic acid equivalents; QE – quercetin equivalents

https://doi.org/10.17221/33/2026-CJFS

Among the onion varieties, purple onion peels exhibited the highest TAC values, followed by red, brown, and white peels. Higher antioxidant capacity was associated with stronger pigmentation, whereas the lower TAC observed in white onion peels was attributed to the absence of pigment-related antioxidant compounds.

Overall, TAC values were higher than those obtained from DPPH and ABTS assays, likely because not all antioxidant compounds effectively react with these radicals, which do not fully represent radicals found in biological systems (Vorobyova et al. 2022). Similar DPPH and ABTS activities for DES and EtOH extracts suggest comparable abilities to neutralise these radicals, while the slightly higher TPC values in EtOH extracts may reflect the selective solubility of certain phenolics in ethanol.

Additionally, all extracts exhibited relatively high TFC values compared with their DPPH and ABTS activities. This may be explained by the predominance of quercetin in onion peels, which shows limited reactivity towards these radicals despite contributing to overall flavonoid content (Vorobyova et al. 2022). Consequently, high TFC values do not necessarily correspond to proportionally higher radical scavenging activity.

Overall, antioxidant activity followed the order: purple > red > brown > white onion peels. This trend is likely related to the higher anthocyanin content in darker-coloured peels, which enhances pigmentation and contributes significantly to antioxidant potential (Zannou and Koca 2022).

Chromatographic analysis showed that quercetin was the predominant flavonoid in onion peel extracts and was detected in all samples except white onion peels. Myricetin was mainly identified in purple onion extracts, while other phenolics such as rutin and kaempferol were below the quantification limit. Although caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, rutin, myricetin, quercetin, and kaempferol standards were included in the HPLC analysis, only myricetin and quercetin could be reliably quantified in the analysed onion peel extracts under the applied chromatographic conditions. The remaining phenolic compounds were either not detected or were present at concentrations below the detection and quantification limits of the method. As shown in Table 9, quercetin content was significantly higher in purple, red, and brown onion peels than in white onion peels, reflecting the greater abundance of flavonoids and anthocyanins in coloured onion varieties.

Although the TPC obtained using EtOH was higher than that obtained with DES (Table 8), the concentration of quercetin in EtOH extracts was nearly two times greater (Table 9). This difference can be explained by the fact that quercetin is not the only flavonoid present in onion peels; other flavonoids and quercetin derivatives also contribute to the TPC.

Previous studies consistently report quercetin and its derivatives as the predominant phenolic compounds in onion peel waste (Stefou et al. 2019). Onion peels contain considerably higher phytochemical concentrations than edible bulb tissues, with quercetin remaining the dominant phenolic compound regardless of onion variety or extraction method (Kumar et al. 2022b). The high phenolic and flavonoid contents obtained in the present study are consistent with these findings and further confirm the strong bioactive potential of onion peel waste as a valuable source of functional compounds.

Anthocyanins and quercetin derivatives are also responsible for colour variations in onion peels, and a positive correlation has been observed between peel colour intensity and phenolic content (Sagar et al. 2022). Accordingly, coloured onion varieties generally contain higher levels of flavonoids and anthocyanins than white onions. For example, red onions may contain substantially higher quercetin concentrations than other *Allium* species, with phenolics particularly enriched in the outer protective layers (Stoica et al. 2023). Similarly, in the present study, darker-coloured onion peel samples exhibited comparatively higher bioactive compound contents, which may be attributed to their naturally elevated anthocyanin and flavonol concentrations.

Several extraction studies further support the abundance of flavonoids in onion peel waste.

Table 9. Myricetin and quercetin amounts under the optimum ultrasound-assisted extraction conditions

Onion peel	Solvent	Myricetin ($mg \cdot g^{-1}$ DS)	Quercetin ($mg \cdot g^{-1}$ DS)
Purple onion	DES	28.4	21.92
Red onion	DES	–	15.4
Brown onion	DES	–	20.84
White onion	DES	–	–
Purple onion	%70 EtOH	20.14	52.36
Red onion	%70 EtOH	–	59.26
Brown onion	%70 EtOH	–	36.13
White onion	%70 EtOH	–	–

DES – deep eutectic solvent; DS – dry sample

https://doi.org/10.17221/33/2026-CJFS

Table 10. Minimum inhibitory concetration (MIC) values of the tested onion extracts for antimicrobial activity[a,b]

ID	Gram-negative bacteria (MIC, μg·mL^{-1})				Gram-positive bacteria (MIC, μg·mL^{-1})				Fungi (MIC, μg·mL^{-1})		
	PA	EC	KP	PM	SA	SE	EF	MRSA	CA	CT	CP
Purple-DES	–	–	–	–	–	156.2	–	–	–	156.2	–
Red-DES	–	–	–	–	–	–	–	–	–	–	–
Brown-DES	–	–	–	–	–	78.12	–	–	–	–	–
White-DES	–	–	–	–	–	78.12	–	–	–	–	–
Purple-EtOH	–	–	–	–	625	312.5	312.5	625	–	78.12	1 250
Red-EtOH	–	–	–	–	625	156.2	156.2	625	625	156.2	1 250
Brown-EtOH	–	–	–	–	625	156.2	39.06	–	625	–	1 250
White-EtOH	–	–	–	–	625	156.2	–	–	625	–	–

[a]reference antimicrobial activity results included 2.44 μg·mL^{-1} ceftazidime for PA, 4.88 μg·mL^{-1} cefuroxime-Na for both EC and KP, 2.44 μg·mL^{-1} for PM, 128 μg·mL^{-1} amikacin for EF, 9.76 μg·mL^{-1} cefuroxime for SE, 1.22 μg·mL^{-1} cefuroxime-Na for SA, 4.88 μg·mL^{-1} clotrimazole for CA, and 0.50 and 1 μg·mL^{-1} amphotericin B for CP and CT, respectively; [b]means > 1 250 μg·mL^{-1} activity; DES – deep eutectic solvent; PA – *Pseudomonas aeruginosa* ATCC 27853; EC – *Escherichia coli* ATCC 25922; KP – *Klebsiella pneumoniae* ATCC 4352; PM – *Proteus mirabilis* ATCC 14153; SA – *Staphylococcus aureus* ATCC 29213; SE – *Staphylococcus epidermidis* ATCC 12228; EF – *Enterococcus faecalis* ATCC 29212; CA – *Candida albicans* ATCC 10231; CT – *Candida tropicalis* ATCC 750; CP – *Candida parapsilosis* ATCC 22019

Pal and Jadeja (2019) reported quercetin, kaempferol, and myricetin as the major flavonoids extracted from red onion peels using a ChCl-based DES system, which yielded higher TFC values than conventional solid–liquid extraction methods. Similarly, Lachman et al. (2003) found that red onions exhibited higher TPC and flavonoid concentrations than yellow and white onions when extracted with aqueous ethanol. The findings obtained in this study also demonstrated the effectiveness of DES-assisted extraction for recovering phenolic compounds from onion peel waste, supporting previous reports that DES systems can improve extraction efficiency compared with conventional solvents.

Overall, variations in phenolic composition among onion peels are influenced by factors such as onion genotype, cultivation conditions, post-harvest handling, and extraction technique, highlighting the importance of both onion variety and solvent selection in determining the bioactive potential of onion peel extracts.

Determination of minimum inhibitory concentrations (MIC). To evaluate differences in antimicrobial activity among onion varieties, the antibacterial and antifungal activities of extracts were assessed *in vitro*. The screening was conducted against four clinically relevant Gram-negative bacterial strains, four Gram-positive bacterial strains, and three fungal strains. Standard antibiotics (ceftazidime, cefuroxime sodium, cefuroxime, and amikacin) and antifungal agents (clotrimazole and amphotericin B) were used as positive controls, as presented in Table 10.

The antimicrobial activity results demonstrated that extracts prepared with 70% EtOH exhibited higher biological potency against several microorganisms compared with those prepared using DES. However, none of the tested onion extracts, including those prepared with 70% EtOH, exhibited antibacterial activity against Gram-negative bacterial strains.

In contrast, DES-based onion extracts showed antibacterial activity only against *Staphylococcus epidermidis*, while no inhibitory effects were observed against the other tested bacterial strains. Conversely, onion extracts prepared with 70% EtOH demonstrated measurable antibacterial activity against several Gram-positive bacterial species, indicating a stronger antimicrobial performance of EtOH extracts in comparison to DES extracts.

Similar trends were observed in the antifungal activity assays. While DES-based extracts from onions of different colour did not exhibit antifungal activity, the 70% EtOH extracts showed moderate to weak inhibitory effects against the tested *Candida* species.

CONCLUSION

This study demonstrates that DES, particularly ChCl: 1,4-but: H_2O, significantly enhanced the extraction of antioxidant compounds from purple onion peels compared with conventional solvents such as EtOH.

https://doi.org/10.17221/33/2026-CJFS

Under the optimised UAE conditions (298 W, 30 min, and a L/S ratio of 100 mL·g^{-1}), purple onion peels exhibited a TAC of 582.96 mg TE·g^{-1} DS and contained substantial amounts of myricetin and quercetin, highlighting their potential as valuable sources of natural antioxidants.

The optimisation of UAE parameters played a critical role in maximising extraction efficiency, while the absence of major flavonoids in white onion extracts further emphasises the importance of onion variety selection. Overall, the findings of this study indicate that purple and red onion peels, particularly when processed using appropriately designed DES systems, represent promising and sustainable raw materials for the development of functional food ingredients and natural antioxidant additives.

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Received: March 3, 2026
Accepted: July 8, 2026
Published online: August 12, 2026