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Research Paper

Study on the Extraction of Polyphenols, Polysaccharides, and Saponins from Camellia oleifera Leaves Using Deep Eutectic Solvents and Their Activities

Scott Billet [1], Martin Duran [1], Nicolas Fisherman [2,*]

[1] Department of Biochemistry and Molecular Biology, East Tennessee State University, Johnson City, TN, 37614, USA
[2] Institute of Agrochemistry and Food Technology- National Spanish Research Council (IATA-CSIC), Valencia, Spain
*Corresponding author: Nicolas.fisherman@iata.csic.es

Abstract. Camellia oleifera Abel., a unique Chinese oil crop of the Theaceae family, supplied the leaves from which polyphenols, polysaccharides and saponins were fractionated with deep-eutectic solvents. COSMO-RS and an artificial neural network were coupled to identify the best deep-eutectic solvents for extracting polyphenols, polysaccharides and saponins from C. oleifera leaves. The main findings are as follows: 1. Polyphenols from *Camellia oleifera* leaves were recovered with ultrasound-assisted deep eutectic solvents (DESs); COSMO-RS modelling guided the selection of the most effective DES to maximise yield. After response-surface tuning of sonication time, power and solvent loading, the ultrasonic protocol delivered 131.63 ± 0.85 mg GAE g^{-1} of polyphenols—twice the haul achieved with classic ethanol soaking. 2. A 9-6-1 ANN, fed with COSMO-RS descriptors, predicted pH and solubility for 36 DES candidates; the choline chloride–sorbitol mixture (ChCl-Sor, pH ≈ 4.5) matched the optimum for protease and cellulase and was chosen for polysaccharide extraction. RSM optimised DES-assisted enzymatic extraction (DES-dEAE): at 33:1 liquid-solid ratio, 3:1 enzyme ratio, 15 min and 40 % water, polysaccharide yield peaked at 21.71 ± 0.21 %. 3. Saponins from *Camellia oleifera* leaves were recovered with ultrasound-assisted deep eutectic solvents (DESs); COSMO-RS modelling guided the selection of the most effective DES to maximise yield. Solid-phase extraction was used to separate saponins from DESs. Preliminary identification showed that saponins from Camellia oleifera leaves include unique saponins such as Oleiferasaponin A3, Sanchakasaponin C, and Sanchakasaponin D. Based on the identification results, COSMO-RS was used to predict the solubility of 36 DESs with six specific saponins. Combined with saponin extraction yield, the DES composed of choline chloride and fructose (ChCl-Fru) was selected as the most suitable DES, with a purified maximum extraction yield of 110.55 ± 0.33 mg TS/g. Experiments demonstrated that the ChCl-Fru group exhibited good antibacterial activity, with significant inhibition against Escherichia coli. The inhibition zone diameter reached 32.87 ± 0.48 mm, superior to the penicillin group, and the growth of E. coli was inhibited at saponin concentrations of 0.78–1.56 mg/mL.

Keywords: *Camellia oleifera; polyphenols; polysaccharides; saponins; DES*

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1 Introduction

Camellia oleifera Abel. is a general term for perennial evergreen shrubs or small trees in the Theaceae family, genus Camellia, with high seed oil content and economic cultivation value. The seeds after removing the exocarp of Camellia oleifera fruit are called Camellia oleifera seeds or tea seeds, and the extracted edible oil is called tea oil. Camellia oleifera is light-loving and suitable for subtropical hilly areas, with an optimal annual average temperature of 14–21°C, sunshine duration of 1,800–2,200 h, relative humidity of 74–85%, and annual precipitation of 800–2,000 mm. It prefers slightly acidic yellow or red soil with pH 4–6, is tolerant of poor soil, and grows best in loose, deep, well-drained, fertile sandy loam, resulting in good fruit set, high yield, and oil content. Camellia oleifera stands alongside oil-palm, olive and coconut as one of the globe's four flagship woody oil crops, dominating hillside plantations across Hunan, Sichuan and Jiangxi. With 73 million hectares under canopy, it is by far China's largest woody source of edible oil.

Polyphenols are natural plant metabolites bearing multiple –OH groups on aromatic rings. The polyphenol family is large and mainly includes flavonoids, phenolic acids, lignans, stilbenes, and tannins. They are key chemical substances for plants to resist UV radiation, pathogens, and herbivores. Plant polyphenols are important active components in human diet and medicine, with significant antioxidant, anti-inflammatory, and disease prevention potential. Yu [1] identified 17 flavonoid components in Camellia oleifera flowers using UHPLC-Q-MS and found that they significantly improved symptoms of hyperglycemia and hyperlipidemia, alleviated oxidative stress, protected liver and pancreatic tissues, and improved insulin resistance effects. Plant polyphenols have wide sources, diverse types, high safety, and advantages of multi-pathway and multi-target actions, making them a research hotspot in fields such as food industry, medicine, and cosmetics.

Polysaccharides are high-molecular-weight carbohydrates in which monosaccharides are linked by glycosidic bonds; they are ubiquitous in plants (cellulose), animals (chitin) and microbes (exopolysaccharides). As one of the most abundant biomolecules in nature, polysaccharides serve basic functions such as structural support and energy storage in organisms. Jiang Zhonggui [2] showed through metabolomics studies that lemon polysaccharide LPs-1a protected Min6 cells under high glucose conditions by regulating histidine metabolism, promoting Min6 cell secretion, and achieving auxiliary hypoglycemic effects. Sun Jin [3] demonstrated that Atractylodes macrocephala polysaccharide alleviated alcohol-induced "gut-liver axis" tissue damage and is a promising prebiotic. Guo Shiwei confirmed that 40 μg/mL Artemisia annua polysaccharide alleviated LPS-induced inflammation and epithelial barrier dysfunction in c-IECs by inhibiting NF-κB and MLCK signaling pathways. The unique biological activities and physical properties of plant polysaccharides make them core research subjects in fields such as medicine, food, and materials.

Saponins are a class of secondary metabolites widely found in plants, consisting of a hydrophobic aglycone (triterpenoid or steroid) linked to a hydrophilic sugar chain via glycosidic bonds. The name originates from their unique surfactant properties—shaking an aqueous solution produces persistent soap-like foam. As natural chemical barriers for plants against pathogens and herbivores, saponins are particularly abundant in medicinal and edible plants such as ginseng, licorice, Panax notoginseng, soybean, and Bupleurum. Based on aglycone structure, plant saponins can be divided into triterpenoid saponins and steroid saponins, both exhibiting significant biological activities and functional diversity. With their multi-target mechanisms, low toxicity, and renewable sources, plant saponins show broad prospects in fields such as innovative drugs, functional foods, green daily chemicals, and agricultural biostimulants, becoming an important bridge connecting traditional plant wisdom and modern biotechnology. Saponin compounds are biogenic, and when analyzing compound structures using mass spectrometry, comparing with existing identification literature allows rapid identification of plant saponin compounds.

The efficient extraction of plant polyphenols, polysaccharides, and saponins directly affects the production value of plants. With the increasing medicinal and economic value of plant polyphenols, polysaccharides, and saponins, exploring more efficient, economical, and environmentally friendly extraction processes has become a major research demand. Plant polyphenols, polysaccharides, and saponins are three important classes of natural active components. As research deepens, their extraction technologies have distinct characteristics and advantages/disadvantages due to differences in chemical properties. Continuous reflux extraction is an efficient and energy-saving technique for natural product extraction. Its core principle is to achieve multiple dynamic extractions of target components through continuous solvent recycling. This method is typically implemented using a Soxhlet extractor. Soxhlet extraction compensates for the high solvent consumption and cumbersome operation of reflux extraction but requires longer time and is unsuitable for heat-sensitive components. Enzyme-assisted extraction (EAE) disrupts plant or microbial cell structures through enzymatic hydrolysis, reducing mass transfer resistance and improving the dissolution efficiency of active components. It is a green extraction technology that uses enzymes to efficiently and specifically degrade cell walls or decompose cell contents, thereby releasing target natural products. EAE has important applications in fields such as plant active component extraction, food and health products, and waste recycling, offering advantages such as high selectivity, mild conditions, environmental friendliness, and significant efficiency enhancement. Currently, limitations such as high cost, complex process optimization, and enzyme residues restrict the application of EAE. Gene editing technology can improve enzyme activity, stability, and reusability [4].

Deep eutectic solvents (DESs) arise from hydrogen-bond pairing of an HBA with an HBD, offering greener, low-

toxicity alternatives to conventional solvents and ionic liquids. Relative to conventional solvents, DESs exhibit low toxicity, ready biodegradability and tunable properties [5]; Zuo [6] exploited ultrasound-assisted DESs to recover luteolin from *Trollius chinensis*, boosting yield by 23 % over 80 % (v/v) ethanol. Ozturk [7] reported that DESs enhance polyphenol yields by vigorously disrupting plant cell walls, while their performance is governed by the choice of HBA and HBD. DESs assembled from primary plant metabolites—sugar alcohols, sugars, amino acids, organic acids, amines and amides—deliver strong solvating power and an intrinsically benign profile for isolating natural products.

Metabolite-based deep eutectic solvents—built from sugars, polyols, amino acids, carboxylic acids and simple amides—combine high dissolution capacity with food-grade safety, making them tailor-made media for extracting bioactive plant metabolites. The principal polyphenols in *Camellia oleifera* leaves were characterised by UHPLC-Q-Tof-MS/MS. Combined with actual polyphenol yield, the optimal DESs were screened. The ultrasound-assisted polyphenol protocol was refined through response-surface optimisation, driving yield to its maximum. Finally, in vitro antioxidant experiments verified that Camellia oleifera leaf polyphenols have good antioxidant activity. RSM was used to optimize the process conditions for DESs-dEAE, further improving the polysaccharide extraction yield. Finally, in vitro antioxidant activity verified that Camellia oleifera leaf polysaccharides have good antioxidant activity. Saponins were recovered from *Camellia oleifera* leaves with ultrasound-assisted DESs and isolated via SPE; unique congeners were then profiled by UHPLC-Q-Tof-MS/MS. Molecular modeling and COSMO-RS were used to screen the optimal DES, improving saponin extraction yield. Finally, antibacterial experiments demonstrated that Camellia oleifera leaf saponins have good antibacterial activity.

2 Experimental Process

2.1 DESs Preparation

DESs were prepared following Dai [8] with minor changes: choline chloride (HBA) and the chosen HBD were mixed 1:1 (mol/mol) at 80 °C until a clear, homogeneous liquid formed. Upon cooling to room temperature the melt remained clear, confirming DES formation [9]; water was then added to 30 % (v/v) to lower viscosity and streamline downstream extraction and clean-up. The HBD pool comprised acids (formic, propionic, lactic, acetic, malonic), alcohols (1,2-propanediol, glycerol, xylitol, ethylene glycol, sorbitol), sugars (glucose, fructose, maltose, sucrose) and amines/amides (urea, methylurea, acetamide, ethanolamine). The HBA was choline chloride (ChCl). All reagents were analytical grade with purity of 99.7%. Eighteen DESs were ultimately prepared: ChCl-FA, ChCl-PA, ChCl-LA, ChCl-AA, ChCl-MA, ChCl-PG, ChCl-Gly, ChCl-Xyl, ChCl-EG, ChCl-Sor, ChCl-Glu, ChCl-Fru, ChCl-Mal, ChCl-Suc, ChCl-Ure, ChCl-Met, ChCl-Ace and ChCl-Eth.

2.2 Extraction and Separation of Polyphenols, Polysaccharides, and Saponins from Camellia oleifera Leaves

Polysaccharides from Camellia oleifera leaves were extracted using Soxhlet extraction. *Camellia oleifera* leaves were oven-dried at 65 °C, ground to powder, and sequentially defatted/de-pigmented with petroleum ether and ethanol [10]. P-CL was then isolated by hot-water, DES-only and enzyme-assisted DES protocols (cellulase + protease) [11]. For DES extraction, ChCl-Sor was stirred with *Camellia oleifera* leaf powder; for enzyme-assisted DES extraction, cellulase and protease were first activated in ChCl-Sor, the powder was then added and the mixture stirred. All three protocols began under identical conditions—45 °C, 40 min, 10:1 liquid-to-solid ratio—and for the enzyme-assisted variant a protease:cellulase ratio of 1:2 was applied. Polysaccharide liquor was shaken with Sevag solvent (n-butanol/chloroform 1:4) at a 1:3 sample-to-solvent ratio to strip off coagulated proteins. The solution was dialysed (3 500 Da, 3 d), evaporated, concentrated and lyophilised; the resulting powders from water, DES and enzyme-DES protocols were coded P-CL-W, P-CL-D and P-CL-E, respectively.

The extraction method for polyphenols from Camellia oleifera leaves was based on Y. Ma et al. [12] with some modifications. Ultrasound-assisted extraction was performed with a KH7200DB unit (ks-hechuang, China): 0.5 g Camellia oleifera powder was treated with 5 mL DES or 80 % (v/v) ethanol. Extraction proceeded at 60 °C and 200 W for 30 min; after centrifugation the supernatant was kept at 4 °C in darkness. Crude polyphenols were cleaned on a C18 SPE cartridge. The cartridge was pre-washed with ethyl acetate followed by 0.35 % (v/v) formic acid; after loading, polar interferents were removed with the same aqueous formic acid, and polyphenols were

recovered with ethyl acetate. Ethyl acetate was evaporated under N_2 at 30 °C and the residue freeze-dried to yield polyphenol powder.

For saponin recovery, 0.5 g Camellia oleifera powder was extracted with 5 mL DES or 80 % (v/v) ethanol. Extraction was run at 60 °C, 200 W, 30 min; the centrifuged supernatant was kept at 4 °C in darkness, and crude saponins were cleaned on an SPE column. The cartridge was pre-conditioned with ethanol followed by 0.35 % (v/v) formic acid; after loading, polar impurities were rinsed with the same aqueous formic acid, and saponins were recovered with ethanol. Ethanol was evaporated under N_2 at 30 °C and the residue freeze-dried to yield saponin powder.

2.3 Determination of Polyphenol, Polysaccharide, and Saponin Content in Camellia oleifera Leaves

Total polyphenols were quantified by the Folin-Ciocalteu assay, adapted from Apak et al. [13], on a Shimadzu UV-1000 spectrophotometer. One-hundred microlitres of 60-fold-diluted crude extract and 50 µL of 10 % (v/v) Folin-Ciocalteu reagent were mixed; 300 µL of 10 % Na_2CO_3 was then added and the mixture kept in darkness for 1 h. Absorbance was read at 750 nm and total polyphenol content was quantified against a gallic-acid calibration curve prepared in 80 % (w/v) ethanol. From each DES family, the mixture releasing the most polyphenols was taken forward; data are expressed as mg gallic-acid equivalents (GAE) per gram dry weight (d.w.).

Total carbohydrate in *Camellia oleifera* leaf polysaccharides was assayed by the phenol–sulfuric acid method [14]: glucose, 6 % phenol and conc. H_2SO_4 were mixed 2:1:5 (v/v/v). After vigorous vortexing, the mixture was boiled for 15 min, cooled, and read at 490 nm; a 0.5 mg mL^{-1} polysaccharide solution was analysed identically.

Saponin content was determined colorimetrically: 0.5 mL sample or tea-saponin standard was pipetted into a 10 mL tube, 0.5 mL 8 % ethanolic vanillin was added, followed by 4 mL 77 % H_2SO_4, and the mixture was heated at 60 °C for 15 min. Following a 10-min ice-water cool-down, absorbance was read at 550 nm against deionized water as blank. A 0.2–1 mg mL^{-1} tea-saponin calibration curve ($R^2 > 0.99$) was used for quantification; yield is reported as mg tea-saponin equivalents (TS) per gram. DESs were ranked by this extraction yield.

2.4 Determination of Antioxidant Activity of Polyphenols and Polysaccharides from Camellia oleifera Leaves

Samples were prepared at 0.1–2 mg mL^{-1}; vitamin C served as positive control and deionized water as blank. One part sugar solution was mixed with eight parts 0.2 mM DPPH in ethanol, held in darkness at room temperature for 15 min, and absorbance read at 517 nm; samples spanned 0.1–2 mg mL^{-1}. Vitamin C was the benchmark and DI water the blank; one volume of sugar solution met 40 volumes of ABTS reagent, stood in the dark for 10 min, then absorbance at 734 nm was logged. Sample window: 0.1–2 mg mL^{-1} ; VC served as reference and DI water as blank; one part sugar solution plus ten parts FRAP reagent was kept dark for 30 min before reading A593. An $FeSO_4$–ethanol series was run in parallel, and 1 mmol L^{-1} Fe^{2+} absorbance was set as one FRAP unit to build the calibration line.

2.5 Determination of Antibacterial Effect of Total Saponin Extract from Camellia oleifera Leaves

Antibacterial activity of the total saponin extract against Bacillus subtilis, Pseudomonas aeruginosa, Salmonella, Staphylococcus aureus and Escherichia coli was evaluated by the agar punch method, measuring inhibition-zone diameters. Specifically, Camellia oleifera saponins were prepared at 4 mg/mL with DMSO, with final DMSO concentration < 0.1%. Frozen experimental bacterial strains were transferred to LB medium for activation. The activated bacterial suspension was diluted to 0.5 McFarland turbidity. Sterile Oxford cups were used to punch three small holes evenly in the center of LB solid medium, with each hole spaced more than 25 mm apart. 0.5 mL of diluted bacterial suspension was evenly spread on the medium surface. Once the agar surface dried, 100 µL of each test solution was pipetted into the wells; 0.1 % DMSO served as the blank and 50 µg mL^{-1} ampicillin sodium as the positive control. Plates were held at 37 °C for 24 h; inhibition diameters were gauged with the cross-streak technique [15].

3 Results and Discussion

DOI:

3.1 Screening of DESs

Being natural and green, DESs are expected to be low-cost, recyclable and tailorable to the target analytes; guided by COSMO-RS, 17 binary DESs were prepared from ChCl and selected HBDs. At a 1:1 HBA:HBD ratio all 17 mixtures formed homogeneous, stable liquids at room temperature, confirming successful DES synthesis. Grouped by HBD type, the 17 DESs were classified as acid-, sugar-, alcohol-, or amine/amide-based and screened for phenolic extraction from C. oleifera leaves. Figure 1a shows that ChCl-PG, ChCl-Eth and ChCl-Ace delivered markedly higher yields than 80 % ethanol (control), aligning with COSMO-RS predictions. TPC values for ChCl-PG and ChCl-Ace were 126.04 and 121.57 mg GAE g^{-1}, respectively—exceeding that of *Ligustrum lucidum* polyphenols (101.46 mg GAE g^{-1}) determined by the same protocol [16]. Top yielders from each class—ChCl-LA, ChCl-PG, ChCl-Fru and ChCl-Ace—were advanced; polyphenols were back-extracted with a C18 cartridge. The purification efficiency of the C18 column for polyphenols is shown in Figure 1b. Additionally, the order of extraction speed was: ChCl-Ace > ChCl-PG > ChCl-LA > ChCl-Fru. Extraction efficiency and polyphenol recovery are governed by the physical properties of the DESs employed [17]. ChCl-PG and ChCl-Ace possess high polarity and moderate viscosity, affording stronger affinity for *C. oleifera* polyphenols than other solvents, yet their elevated viscosity somewhat limits extraction efficiency [18]. Despite its carboxyl groups, García Roldán's work indicates that DESs bearing carboxylic-acid HBDs can react with cell walls at elevated temperatures, boosting wall disruption [19]. Yet their weaker polyphenol affinity, lower polarity and reduced pH diminish yield; balancing overall efficiency and SPE elution speed, ChCl-Ace was chosen for process optimisation.

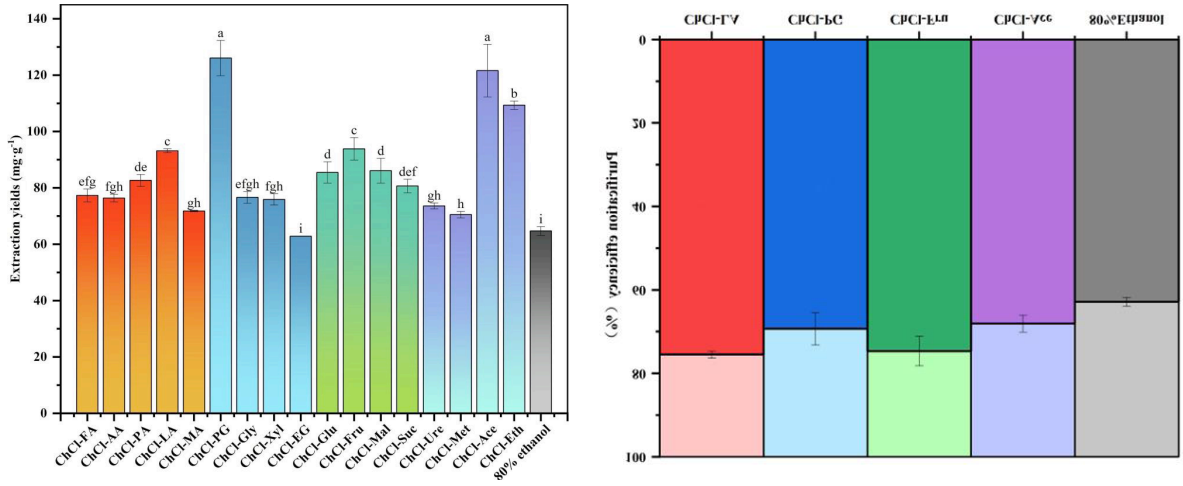

Figure 1 The extraction rates of polyphenols from C. oleifera leaves using 17 DESs (a); The purification efficiency of C18 column for four types of DESs (b)

3.2 Quantitative Analysis of Polyphenols

Table 1 Quantitative results of the 6 polyphenolic compounds in *C. oleifera* leaves

Compounds	ChCl-LA	ChCl-Prop	ChCl-Fru	ChCl-Ace	80% Ethanol
p-coumaric acid	20.41 ± 1.45				
Isoquercetin	251.29 ± 8.90a	56.41 ± 2.56c	65.89 ± 1.20cd	188.24 ± 0.99b	45.35 ± 0.69e
Rutin	157.99 ± 4.21a	43.36 ± 1.20c	55.73 ± 0.46a	163.25 ± 12.79b	11.19 ± 1.42e
Nicotiflorin	147.69 ± 8.11a	70.95 ± 4.12c	42.83 ± 0.03b	91.52 ± 7.17d	
Afzelin	837.02 ± 20.13a	67.19 ± 1.01c	41.15 ± 1.04b	194.97 ± 8.25d	5.42 ± 0.26e
Quercetin	-	19.38 ± 2.18b	-	24.52 ± 2.42a	-

Six reference polyphenols were quantified in the selected DESs versus 80 % ethanol (Table 1); both individual yields and the spectrum of compounds recovered exceeded those of the ethanol control, mirroring the total-polyphenol data.Among the four DESs, ChCl-LA delivered the highest afzelin yield (837 μg g^{-1}), 4- to 20-fold greater than the other systems. Except for afzelin, the extraction yields of the other three components—isoquercetin, p-coumaric acid, and nicotiflorin—also matched the COSMO-RS calculation results. ChCl-LA and ChCl-Ace pushed yield upward, whereas ChCl-PG and ChCl-Fru slid downward, confirming the in-silico forecast. p-Coumaric acid and quercetin, present only in trace amounts, appeared solely in selected DES extracts.

3.3 Determination of Antioxidant Activity of Polyphenols

DPPH• is the benchmark radical for gauging the scavenging capacity of plant polyphenols. Figure 2a shows that all four DES extracts out-performed the 80 % ethanol control; at 0.4 mg mL^{-1} ChCl-Ace achieved a maximum scavenging rate of 85.9 % versus only 8.4 % for ethanol. ChCl-Ace exhibited the strongest activity (IC_{50} 0.14 mg mL^{-1}), whereas ChCl-LA was least effective (IC_{50} 0.75 mg mL^{-1}), in line with Gandhi et al. [20]. The 80 % ethanol extract scavenged only 43.4 % at 2 mg mL^{-1}; Figure 2b reveals stage-dependent differences in $ABTS^{\cdot+}$ quenching among the groups. Overall, all four DES extracts surpassed the 80 % ethanol control, with ChCl-Fru the most potent; at 2 mg mL^{-1} it achieved 77.9 % scavenging—approaching vitamin C (90.5 %) and far exceeding ethanol. IC_{50} values were 0.39 mg mL^{-1} (ChCl-Fru), 1.17 mg mL^{-1} (ChCl-Ace) and 1.17 mg mL^{-1} (ChCl-PG), confirming that DES-extracted polyphenols outperform ethanol-extracted ones in radical scavenging. Figure 2c shows that DES-extracted polyphenols displayed uniformly strong iron-reducing power, whereas the ethanol extract exhibited the lowest antioxidant capacity. The pattern mirrors DPPH· and $ABTS^{\cdot+}$ data, implying that high-antioxidant polyphenols dominate the DES-extracted fractions. Dabetic et al. [21] reported superior antioxidant activity for DES-extracted grape-seed polyphenols versus aqueous extracts; nevertheless, below 1 mg mL^{-1} the 80 % ethanol extract matched or even exceeded the activity of several DES extracts. Above 1.7 mg mL^{-1} the activities of ChCl-Ace, ChCl-LA and ChCl-Fru surpassed vitamin C, demonstrating pronounced in vitro antioxidant capacity for DES-extracted polyphenols.

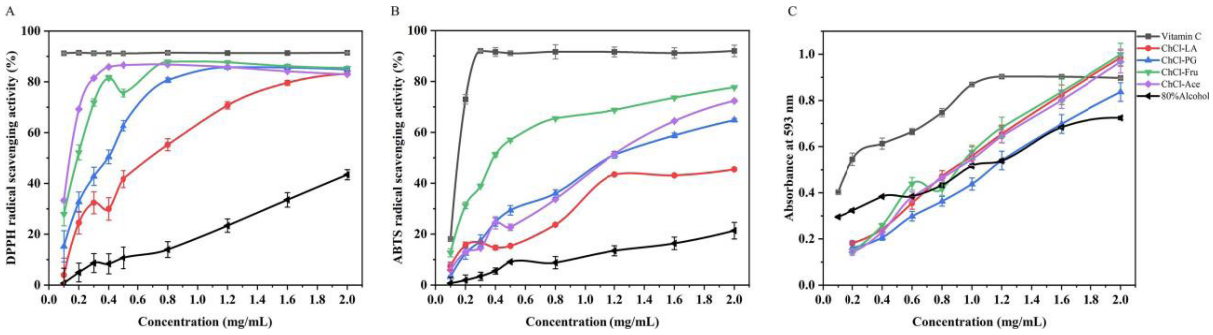

Figure 2 The antioxidant activities of C. oleifera leaves polyphenols at different concentrations

Note: A, DPPH· scavenging abilities, B, ABTS·+ scavenging abilities, and C, Ferric reducing ability of plasma

3.4 Determination of Physicochemical Characteristics of Polysaccharides

Table 2 summarises yield, chemical make-up and physical traits, revealing that the extraction protocol markedly shapes crude-polysaccharide composition. Enzyme-assisted DES extraction proved most efficient, affording 19.1 % polysaccharide with elevated total carbohydrate (78.3 %) and lowered protein content. Crude polysaccharide contained 13.6 % protein, falling to 4.0 % after purification—lower than both counterparts—confirming the enzyme-DES route as the most efficient and highest-quality option. Total starch was <1.6 % in all samples, confirming low contamination; the broad solubilising power of DESs also co-extracts minor components, so summed values fall short of 100 %. Post-purification analyses showed complete DES removal with no residual salt.

Table 2 Yield, chemical constituents and physical properties of P-CLs

	P-CL-W	P-CL-D	P-CL-E
Polysaccharide yield (%)	10.26 ± 0.10c	13.63 ± 0.13b	19.1 ± 0.22a
Total carbohydrate content (%)	67.51 ± 0.66c	74.53 ± 1.45b	78.27 ± 1.81a
Proteins content (%)	20.15 ± 1.57b	23.17 ± 1.98a	4.02 ± 0.62c
Uronic acid content (%)	3.58 ± 0.23c	8.64 ± 0.55b	9.45 ± 0.60a
Total starch content (%)	1.64 ± 0.06a	0.82 ± 0.0b	0.39 ± 0.02c
Viscosity (mm2/s)	0.92 ± 0.01b	0.96 ± 0.01a	0.93 ± 0.02b

As shown in Figure 3a, Camellia oleifera leaf polysaccharides exhibit typical polysaccharide infrared spectra. FTIR showed O–H stretch (~3366 cm^{-1}), C–H stretch (~2931 cm^{-1}), asymmetric C=O (~1620 cm^{-1}), carboxyl O–H (~1421 cm^{-1}), pyranoid C–O–H and C–O–C linkages (~1088 and ~1041 cm^{-1}), plus weak β-glycosidic/pyran fingerprints at 600–800 cm^{-1}. The ~1620 cm^{-1} band reflects amino-acid backbone vibration; its low intensity at 1620.27 cm^{-1} in P-CL-W confirms minimal protein contamination.

3.5 Determination of Antioxidant Activity

Natural polysaccharides are attractive antioxidant agents: although less potent than small-molecule antioxidants (polyphenols, carotenoids, vitamins), they donate H-atoms or transfer electrons to stabilise radicals and display notable in vitro activity.

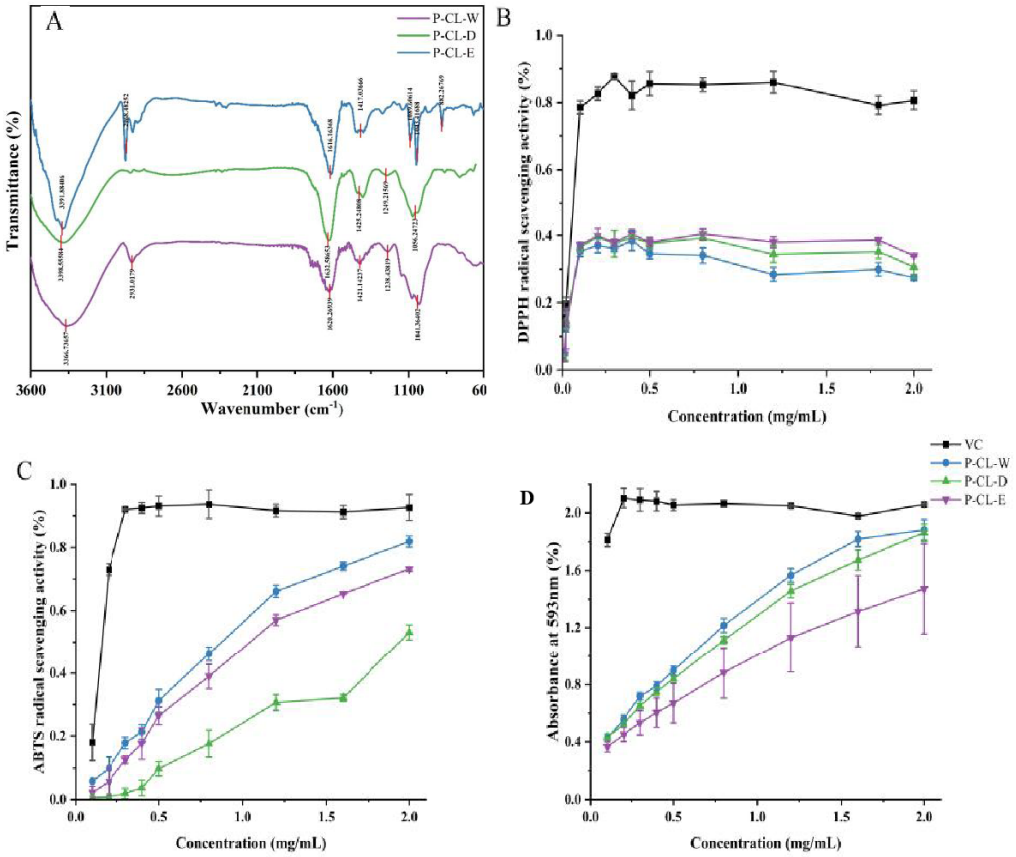

Figure 3 Infrared spectra of P-CLs (a); The antioxidant activities of P-CLs at different concentrations

After purification to exclude non-carbohydrate antioxidants, DPPH·, ABTS·$^{+}$ and FRAP assays were applied. Figure 3b shows that all three C. oleifera polysaccharide preparations scavenge DPPH· radicals. Scavenging plateaus once polysaccharide concentration hits 0.1 mg mL^{-1}. At this level P-CL-E achieved 37.5 % scavenging, statistically on par with the other preparations; literature values for P-CL-W at 2 mg mL^{-1} (≈35 %) corroborate these findings. Figure 3c reveals that P-CL effectively quenches ABTS·$^{+}$ radicals in a concentration-dependent manner. At 2 mg mL^{-1}, ABTS·$^{+}$ scavenging reached 82.5 % (P-CL-W), 51.3 % (P-CL-D) and 73.2 % (P-CL-E), giving IC_{50} values of 0.83, 2.05 and 1.04 mg mL^{-1}; thus P-CL-W > P-CL-E > P-CL-D in activity. This outcome likely reflects the lower purity of P-CL-W, whose residual small-molecule antioxidants boost ABTS·$^{+}$ scavenging beyond that of the cleaner preparations. Figure 3d shows that all three P-CL preparations possess concentration-dependent total antioxidant capacity; at any given level P-CL-W and P-CL-D are essentially equivalent and outperform P-CL-E. This pattern mirrors enzyme-assisted extraction: P-CL-E exhibits unstable, concentration-proportional iron-chelating activity, implying that enzymatic cell-wall hydrolysis leaves the C. oleifera wall structure labile. Optimising a more robust enzymatic hydrolysis regime remains promising; collectively, P-CL shows clear potential as a natural antioxidant agent.

3.6 Screening of DESs and Separation Efficiency of C18 Column

Grouped by HBD type into acid, sugar, alcohol and amine/amide classes, the 17 DESs were screened for saponin recovery from *C. oleifera* leaves (Fig. 4a); every NADES out-yielded the 80 % ethanol control. Among them, ChCl-PG and ChCl-EG had the highest extraction yields. However, amine and amide-based NADESs had the worst extraction efficiency, contradicting COSMO-RS predictions. Yield swings stem from a mix of viscosity, polarity and other solvent traits that shift together as the DES recipe changes. Notably, the extraction yields of fructose and sucrose NADESs were abnormally higher than all extraction solvents, clearly indicating false positives. The crude extract was not purified, and sugars underwent caramelization reaction with concentrated sulfuric acid at high temperature, generating brown substances that affected the test results. Therefore, ChCl-LA, ChCl-PG, ChCl-Fru, and ChCl-Ace with better extraction effects from the four NADES categories were selected for purification to determine the actual extraction yield.

Saponins were isolated from DESs on a C18 cartridge; recovery efficiency is depicted in Fig. 4b. The figure shows that Camellia oleifera saponins after column purification have varying degrees of loss, but the overall trend is similar to crude saponins. The actual extraction yield of ChCl-Fru is slightly higher than ChCl-PG, so ChCl-Fru was selected as the optimal DES for Camellia oleifera saponins and used in subsequent experiments.

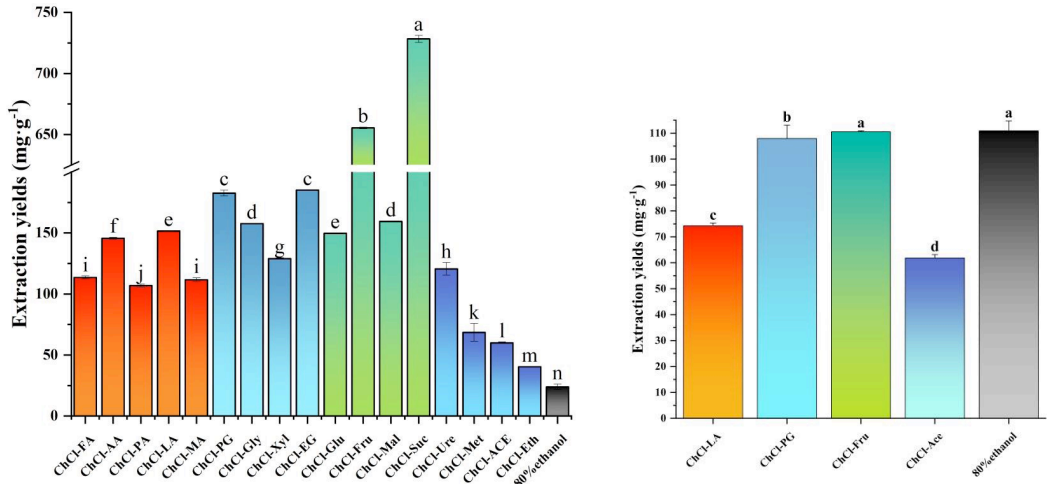

Figure 4 The extraction yields of saponins from C. oleifera leaves using 17 DESs (a); Saponins extraction yield after purification (b)

3.7 Determination of Antibacterial Activity of Camellia oleifera Leaf Saponins

Figure 5 show that 4 mg mL^{-1} Camellia oleifera leaf saponins curb colonies of E. coli, B. subtilis, S. aureus, P. aeruginosa and Salmonella. Compared to alcohol-extracted Camellia oleifera leaf saponins, ChCl-Fru extracted

saponins have better antibacterial activity. The inhibitory activity against E. coli is particularly significant. The inhibition zone diameter of ChCl-Fru extracted saponins reached 32.87 ± 0.48 mm, larger than the penicillin group, while alcohol-extracted saponins showed no obvious inhibition zone.

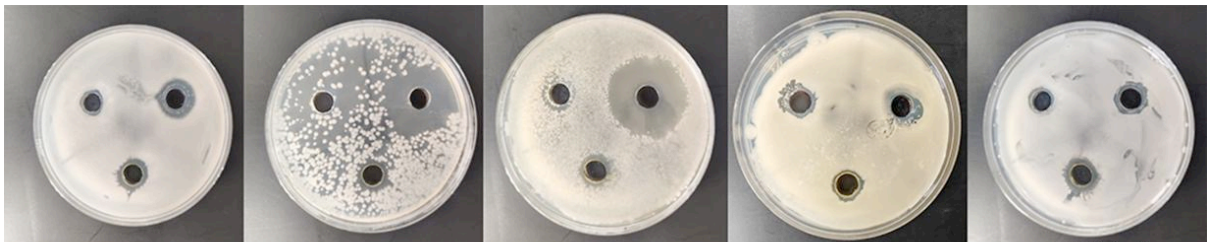

Figure 5 Cycircle igure of C. oleifera leaves saponins （From left to right, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomondium aeruginosa, Salmonella）

Camellia oleifera leaves, as an important plant resource, have significant complexity and diversity in their active component composition. Scientifically and reasonably selecting extraction methods and solvent systems is decisive for improving the extraction efficiency of target active components. This study established differentiated targeted extraction strategies based on the heterogeneity of molecular weight distribution, polarity characteristics, and solubility of polyphenols, saponins, and polysaccharides in Camellia oleifera leaves. Modern phytochemical research shows that synergistic extraction modes combining multiple technologies can overcome the limitations of single methods. Among them, deep eutectic solvents, with their designable physicochemical properties, exhibit unique adaptability advantages in composite extraction systems. Targeting the subcellular distribution characteristics of active components in Camellia oleifera leaves, this study developed a site-specific graded extraction scheme.

Polyphenols and saponins in Camellia oleifera leaves are mainly located in the cytoplasmic matrix of leaf cells. Ultrasound-assisted extraction technology was used to disrupt cell wall structures through cavitation-induced mechanical stress, promoting the outflow of intracellular polyphenols and saponins, effectively improving their extraction yield. Simultaneously, based on the directional extraction utility of DESs, cytoplasmic contents were fully dissolved, and the optimal DESs for polyphenols and saponins, ChCl-Ace and ChCl-Fru, were selected, with extraction yields more than twice that of 80% ethanol extraction. For cell wall-bound polysaccharides, an innovative dual-enzyme synergistic-DESs combined extraction system was constructed. A strategy using cellulase and protease effectively deconstructed the cell wall network structure, releasing encapsulated polysaccharide molecules. To avoid inhibition of enzyme activity by DESs, starting from the optimal pH of enzymes, the solvent's pH was predicted through quantum chemical calculations to assist DES screening. Finally, ChCl-Sor was selected as the extraction solvent, as its pH buffering capacity maintains the stability of the enzymatic hydrolysis system, increasing the polysaccharide yield to 19.1 ± 0.22%, 1.9 times that of traditional hot water extraction. Furthermore, polysaccharides extracted by DES-dEAE have more stable structures at the microscopic level and better thermal stability, indicating broader application potential. This study provides a multi-scale solution for the precise extraction of complex plant components, demonstrating the broad application potential of DESs-technology coupled systems in the field of natural product separation.

The physical characteristics of DESs are the main factors affecting the extraction of polyphenols, polysaccharides, and saponins from Camellia oleifera leaves. This study focused on measuring the viscosity, conductivity, polarity, and pH of DESs. Viscosity mainly affects the speed of extraction and separation processes, while polarity and pH mainly affect the extraction yield of active substances. In polyphenol extraction experiments, the viscosity, conductivity, and pH of four main DESs were measured. It was found that viscosity and the speed of polyphenol purification and DES recovery have a one-to-one correspondence. DESs with high viscosity, such as ChCl-Fru with viscosity of 26.60 ± 0.02 mm^2/s, more than twice that of other DESs, require correspondingly more time during SPE extraction of polyphenols. Therefore, based on practical production considerations, viscosity is an important factor in screening DESs.

pH guided DES screening in the coupled system. In the enzyme-assisted DES extraction of polysaccharides, pH

was a key guiding factor. Although the adaptive pH range of enzymes is broad, in a system requiring coexistence of two enzymes, the solution pH must meet the optimal pH of both enzymes; otherwise, it cannot be determined whether one enzyme has normal activity. Thus, the optimal pH of the solution was fixed to a relatively small range. This experiment used ANN to accurately predict the pH of DESs, thereby screening ChCl-Sor as the optimal DES.

To further explore the compound composition of active substances in Camellia oleifera leaves and meet the needs of QSPR model construction for molecular structure determination, this study used liquid chromatography-mass spectrometry to extensively identify monomeric small molecules in Camellia oleifera leaves. A total of 50 polyphenols and 10 saponins were identified, among which Oleiferasaponin A3, Oleiferasaponin D4, Sanchakasaponin C, Sanchakasaponin D, and Yuchasaponin B are unique saponin compounds of Camellia oleifera with application potential. Substances with high content and high biospecificity were selected for precise QSPR modeling and used to represent the prediction of total polyphenol and saponin yields. Through extraction yield determination and quantitative analysis, this strategy has significant advantages, greatly reducing computational requirements while accurately predicting extraction yield and assisting in screening relatively optimal extraction solvents. For an effective component, dozens of DESs are typically screened, not only for HBA and HBD but also considering the ratio of components. Pavlić [22] et al. studied 20 NADESs to improve the polyphenol yield of wild thyme. Using COSMO-RS to predict solubility is an efficient screening method. The monosaccharide composition of Camellia oleifera leaf polysaccharides was determined using liquid chromatography. Based on the monosaccharide composition and the dynamic changes in physicochemical indicators after monosaccharide synthesis into polysaccharide chains, the solubility of Camellia oleifera leaf polysaccharides in DESs was predicted using precise QSPR models for monosaccharides, effectively assisting in screening the relatively optimal DES for polysaccharides. Simultaneously, a 9-6-1 ANN model was constructed to link the QSPR of DESs with their pH mathematically. The model's $R^2 > 0.99$, indicating good predictive performance. This model predicts the pH of DESs by describing molecular surface charge models, successfully predicting the pH of 36 DESs. Based on the optimal pH of enzymes, two enzymes (protease and cellulase) with similar optimal pH ranges were selected, setting pH 4.5 as the condition for optimal DES selection. Combined with the predicted solubility of DESs with monosaccharides, the screening of the optimal DES for polysaccharides was completed, ChCl-Sor as the optimal DES. This study successfully started from the optimal pH of enzymes, used pH as an important screening condition for DESs, and ultimately established a DESs and dual-enzyme coexistence extraction system. Different from traditional DES model construction in the COSMO-RS system, where DESs are treated as mixtures of HBA and HBD solvents, this study constructed hydrogen-bonded molecules of HBA and HBD during model construction. Using molecular model optimization modules, DES molecules were artificially synthesized at the molecular level based on the lowest energy state principle. Then, the molecules were solvated, meaning HBA and HBD were fused at the molecular modeling stage. In the COSMO-RS system, DESs are treated as a single solvent for direct extraction of active substances from Camellia oleifera leaves. The advantage of this method is that it fully reflects the significant influence of hydrogen bonds on DES solvent properties in real DES solvents, and the prediction results are closer to reality. Finally, the solubility of polyphenols, polysaccharides, and saponins from Camellia oleifera leaves in DESs was successfully predicted, and the optimal DESs were selected for each. However, for a complex extraction system, single-factor prediction accuracy is limited for the entire extraction yield prediction result. More factors need to be considered in simulated extraction systems to achieve better prediction results.

Traditional solvents such as ethanol extraction and water extraction of plant polyphenols, polysaccharides, and saponins usually have good biological activity. This study used DESs as solvents to extract polyphenols, polysaccharides, and saponins from Camellia oleifera leaves, with alcohol extraction and water extraction as controls, to explore the advantages of DES extraction. DES-extracted small molecules such as polyphenols and saponins have better antioxidant and antibacterial activities because DES extraction yields higher content of polyphenols and saponins and can target single compounds with better activity. Compared to alcohol-extracted saponins, DES-extracted saponins clearly have better antibacterial activity, especially against E. coli, with an inhibition zone diameter of 32.87 ± 0.48 mm, far higher than the alcohol extraction group and the penicillin control group. The results indicate that Camellia oleifera leaf polyphenols and saponins have good biological activity, laying a solid foundation for practical applications. Similar antioxidant profiles among the differently extracted polysaccharides stem from their complex but comparatively uniform structures, in contrast to the more compositionally variable polyphenols and saponins. The work confirms that DES-dEAE-extracted polysaccharides

retain strong radical-scavenging power, underpinning the case for deploying Camellia oleifera leaf polymers as natural antioxidants.

4 Conclusion

This study used Camellia oleifera leaves as the research object and employed bioinformatics means based on compound identification to design customized coupled extraction systems for polyphenols, polysaccharides, and saponins from Camellia oleifera leaves. Specifically, customized DESs were screened for different extraction targets, significantly increasing the extraction yield of active substances. Finally, the activities of polyphenols, polysaccharides, and saponins from Camellia oleifera leaves were determined and evaluated to verify that DES-extracted active substances still have high activity.

The main research conclusions are as follows:

(1) For polyphenols from Camellia oleifera leaves, this paper quantified six key polyphenolic compounds, measured the physical characteristics of the four DESs, and comprehensively screened ChCl-Ace as the optimal extraction solvent for polyphenols. Polyphenol release was pushed to 131.63 ± 0.85 mg GAE per gram dry leaf once the extraction window was tightened. The isolated polyphenols proved to be powerful radical scavengers.

(2) For polysaccharides from Camellia oleifera leaves, this study constructed a coupled extraction system of dual enzymes and DESs, screening ChCl-Sor as the optimal extraction solvent for polysaccharides. The in vitro antioxidant activity of polysaccharides was determined.

(3) For saponins from Camellia oleifera leaves, this study constructed a coupled extraction system of ultrasound-assisted DES extraction, comprehensively screening ChCl-Fru as the optimal extraction solvent for saponins. Additionally, the antibacterial activity of saponins was verified, showing that ChCl-Fru extracted saponins have good antibacterial activity

References

[1] Yang P, Li X, Liu Y L, et al. Two triterpenoid glycosides from the roots of Camellia oleifera and their cytotoxic activity [J]. Journal of Asian Natural Products Research, 2015, 17(8): 800-807.

[2] Zhang Z Y, Wu J P, Gao B B, et al. Two new 28-nor-oleanane-type triterpene saponins from roots of Camellia oleifera and their cytotoxic activity[J]. Journal of Asian Natural Products Research, 2016, 18(7): 669-676.

[3] Wu J P, Kang N X, Zhang M Y, et al. Oleiferoside W from the roots of Camellia oleifera C. Abel, inducing cell cycle arrest and apoptosis in A549 cells[J]. Journal of Asian Natural Products Research, 2018, 20(8): 793-806.

[4] Li X, Zhao J, Li X-R, et al. New triterpenoid glycosides from the roots of Camellia oleifera Abel.[J]. Helvetica Chimica Acta, 2015, 98: 496-508.

[5] Saha S K, Dey S, Chakraborty R. Effect of choline chloride-oxalic acid based deep eutectic solvent on the ultrasonic assisted extraction of polyphenols from Aegle marmelos[J]. Journal of Molecular Liquids, 2019, 287: 110956.

[6] Yin C, Liu X, Guo W, Lin T, Wang X, Wang L. Prediction and application in QSPR of aqueous solubility of sulfur-containing aromatic esters using GA-based MLR with quantum descriptors[J]. Water Research, 2002, 36(12):2975-2982.

[7] Toropov A A, Toropova A P. QSPR/QSAR: state-of-art, weirdness, the future[J]. Molecules, 2020, 25(6): 1292.

[8] Li X, Zhao J, Peng C, et al. Cytotoxic triterpenoid glycosides from the roots of Camellia oleifera[J]. Planta Medica, 2014, 80(7): 590-598.

[9] Munjal N S, Sharma M, Singh T R. Development of QSPR Strategy for the Solubility Prediction[J]. Current Computer-Aided Drug Design, 2018, 14(4): 302- 309.

[10] Sugimoto S, Chi G, Kato Y, et al. Medicinal Flowers. XXVI. structures of acylated oleanane-type triterpene oligoglycosides, yuchasaponins A, B, C, and D, from the flower buds of Camellia oleifera-gastroprotective, aldose reductase inhibitory, and radical scavenging effects[J]. Chemical & Pharmaceutical Bulletin, 2009, 57(3): 269-275.

[11] Di T M, Yang S L, Du F Y, et al. Oleiferasaponin A_2, a Novel Saponin from Camellia oleifera Abel. Seeds, Inhibits Lipid Accumulation of HepG2 Cells Through Regulating Fatty Acid Metabolism[J]. Molecules, 2018, 23(12): 3296.
[12] Tu W, Bai L, Zeng S, et al. An ionic fragments contribution-COSMO method to predict the surface charge density profiles of ionic liquids[J]. Journal of Molecular Liquids, 2019, 282: 292-302.
[13] Zuo J, Ma P, Geng S, et al. Optimization of the extraction process of flavonoids from Trollius ledebouri with natural deep eutectic solvents[J]. Journal of Separation Science, 2022, 45(3): 717-727.
[14] Ozturk B, Parkinson C, Gongzalez-miquel M. Extraction of polyphenolic antioxidants from orange peel waste using deep eutectic solvents[J]. Separation and Purification Technology, 2018, 206: 1-13.
[15] Zhou H, Wang C Z, Ye J Z, et al. New triterpene saponins from the seed cake of Camellia Oleifera and their cytotoxic activity[J]. Phytochemistry Letters, 2014, 8: 46-51.
[16] Fu H Z, Wan K H, Yan Q W, et al. Cytotoxic triterpenoid saponins from the defatted seeds of Camellia oleifera Abel[J]. Journal of Asian Natural Products Research, 2018, 20(5): 412-422.
[17] Di T M, Yang S L, Du F Y, et al. Cytotoxic and hypoglycemic activity of triterpenoid saponins from Camellia oleifera Abel. seed pomace[J]. Molecules, 2017, 22(10): 1562.
[18] Zhao Y-M, Wang J, Wu Z-G, et al. Extraction, purification and anti-proliferative activities of polysaccharides from Lentinus edodes[J]. International Journal of Biological Macromolecules, 2016, 93: 136-44.
[19] Rajha H N, Mhanna T, EL Kantar S, et al. Innovative process of polyphenol recovery from pomegranate peels by combining green deep eutectic solvents and a new infrared technology[J]. LWT-Food Science and Technology, 2019, 111: 138-146.
[20] Dehkordi F, Sobati M A, Gorji A E. New molecular structure based models for estimation of the CO2 solubility in different choline chloride-based deep eutectic solvents (DESs)[J]. Scientific Reports, 2023, 13(1): 8495.
[21] Zhang X F, Han Y Y, Bao G H, et al. A new saponin from tea seed pomace (Camellia oleifera Abel) and its protective effect on PC12 cells[J]. Molecules, 2012, 17(10): 11721-11728.
[22] Cysewski P, Jelinski T, Przybylek M. Application of COSMO-RS-DARE as a tool for testing consistency of solubility data: case of coumarin in neat alcohols[J]. Molecules, 2022, 27(16): 5274.