Research Article

INDONESIAN SCHOLAR SOCIETY

Indones. J. Chem. Stud.
2026, 5(1), 20–30
Available online at journal.solusiriset.com
e-ISSN: 2830-7658; p-ISSN: 2830-778X

Indonesian Journal of Chemical Studies

Optimization of Green Extraction Methods and Characterization of Luteolin Particle Size from Celery (*Apium graveolens*) NaDES Ultrasonics for Drug Raw Material Independence

Rizky Yulion Putra[1]*, Ruri Putri Mariska[1], Putri Ningrum Nurin Latifah[1]

[1]*Departement of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi, Jambi City 36122, Indonesia*

Received: 30 Nov 2025; Revised: 11 Jan 2026; Accepted: 27 Jan 2026;
Published online: 12 Jun 2026; Published regularly: 30 Jun 2026

Abstract— The development of luteolin extraction technology using NaDES (Natural Deep Eutectic Solvent) with celery (Apium graveolens) samples will result in technological advances in the self-sufficiency of medicinal raw materials derived from local natural resources. The objective of this research is to develop extraction method for luteolin compounds and to characterize the particle size nanoparticle extracts. Choline chloride is utilized as an HBA, lactic acid, glycerol, and glucose as HBDs. These substances are employed in combinations of 1:1, 1:3, and 1:5. Ultrasonic-assisted extraction (UAE) was performed with variations in time (15, 30, 60 minutes) and temperature (30, 50, 70°C). The %yield of luteolin compound was identified by UV-Vis spectrophotometer. Liquid chromatography-mass spectrometry (LC-MS/MS) was utilized to characterize each compound NaDES extract. Characterized particle size and zeta potental of each NaDES extract was using a particle size analyzer (PSA). The optimal %yield of 1.2468% was achieved through choline chloride-lactic acid (1:3) at 60 minutes and a temperature of 70°C. The characterization of luteolin 3'-methyl ether 7-malonylglucoside and luteolin 7-primeveroside compounds was successfully identified in apium graveolens NaDES extract. Characterization of the particle size and zeta potential values of each group of choline chloride–lactic acid, choline chloride–glycerol, and choline chloride–glucose were 292.2 nm, 428 nm, and 198.3 nm, respectively, with corresponding potential values of 167 mV, 133.9 mV, and 71 mV. Celery extract considerable potential for further development. Obtaining samples is a simple process, and the extract exhibits significant promise in supporting Indonesia's autonomy for domain of medicinal raw materials.

Keywords— *Apium graveolens; Liquid chromatography-mass spectrometry (LC-MS/MS); Natural Deep Eutectic Solvent (NaDES); Particle Size Analizer (PSA); Zeta potential*

1. INTRODUCTION

Conventional extraction methods are still employed as solvents in the food, cosmetics, pharmaceutical, and traditional medicine industries [1,2]. Conventional solvents, such as ethanol, methanol, chloroform, n-hexane, and ethyl acetate, which are frequently utilized in extraction processes, exhibit a tendency to be poorly biodegradable, flammable, and highly toxic [3]. The development of more targeted natural substance extraction technology has led to the emergence of natural deep eutectic solvents (NaDES) as a potential alternative to conventional solvents [4,5]. The study of compound characterization and particle nanoscale dimensions presents a range of opportunities for further formulation studies for targeted therapy. Specifically, the nanoscale size of particles has the potential to enhance the effectiveness of candidate drug ingredients for therapeutic purposes [6–8]. In accordance with the findings of preceding studies, the team has effectively identified luteolin compounds in celery samples. These samples have been found to contain luteolin (4), In this research design, the team will optimize NaDES-based extraction results using acid derivatives, glucose derivatives, and natural alcohol solvents. The research design is expected to support the following objectives: determination % yield luteolin compound and characterization NaDES extraction of celery (Apium graveolens).

NaDES (natural deep eutectic solvents) function as natural eutectic solvents in green extraction processes [9–11] The material's biodegradability is a factor that will contribute to its decomposition in the environment [12]. The NaDES solvent synthesis process entails the amalgamation of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) components, resulting in the formation of hydrogen bonds with a customizable molar ratio [13–15]. The NaDES solvent synthesis process is characterized by its low temperature operation, typically below 100°C, and its relatively brief processing times [4].

***Corresponding author**.
Email address: rizkyyulionputra10@gmail.com
DOI: 10.55749/ijcs.v5i1.117

UV-Vis spectrophotometers are effective instruments The objective of this study is to analyze target compounds and identification of % yield results [16,17]. The NaDES ultrasonic method has been demonstrated to enhance cell permeability through an extraction process that is brief in duration and conducted at ambient temperature. This process has been shown to improve the effectiveness of the yield[18]. It has been demonstrated that superior results can be achieved when using alternative solvents in place of methanol/ethanol [19–21]. The LC-MS/MS instrument provides accuracy in characterizing the compounds extracted from green NaDES [4]. It is imperative to furnish data concerning the types of compounds that were successfully extracted [22] The characterization of particle size is achieved through the utilization of a Particle Size Analyzer (PSA) instrument, in conjunction with a study of zeta potential values. This methodological approach provides a comprehensive assessment of particle size and stability [8,23]. The results of the NaDES extract are presented herein.

Celery (Apium graveolens) has health benefits [24] containing luteolin compounds [25–27] with antimicrobial bioactivity [28], anti-inflammatory [29], anti-cancer [27], [30–32], antioxidants [26] anti-viral [32] anti-diabetes and anti-obesity [25] Choline chloride was selected as the HBA, and lactic acid, glucose, and glycerol were selected as the HBDs because they are part of three types of HBA categories: acids, sugar/glucose derivatives, and natural alcohol derivatives. These categories have been proven to be effective as solvents for the extraction of bioactive compounds [5,18,20,33-36].

2. EXPERIMENTAL SECTION

2.1. Materials

The research was conducted at the Pharmaceutical Biology Laboratory at the Sekolah Tinggi Ilmu Kesehatan Harapan Ibu Jambi from June to September of 2025. The materials used in this research were Celery (*Apium graveolens*), luteolin, choline chloride, lactic acid, glucose, glycerol, demineralized water, DMSO (Merck™), acetonitrile (Merck™), ammonium formate(Merck™), and formic acid (Merck™).

2.2. Instrumentations

The research was conducted in an experimentally. The present study encompassed the collection of plant specimens, their subsequent identification, the extraction of natural compounds, and the analysis of these compounds using a LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry) (Waters™), Particle Size Analyzer (PSA)-zeta potential (Microtrac™), and UV-Vis spectrophotometer (Shimazu™). The apparatus used in this research were cuvette, sonicator, homogenizer/vortex mixer, glass bottle, Erlenmeyer flask, beaker, stirring rod, Whatman filter paper, funnel, centrifuge, Masslynx, MSConvert, and Sirius.

2.3. Sample Determination

The sample used was a portion of soybeans obtained from Jambi. The determination of plant samples was carried out at the Taxonomy Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Indonesia.

2.4. Synthesis of Solvents and Green Extraction with NaDES Ultrasonics

Luteolin extraction was performed from celery (Apium graveolens) samples by optimizing the NaDES-based ultrasonic method. [18,33,37,38]. The components of NaDES were weighed according to the molar ratio of HBA (choline chloride). In order to synthesize a homogeneous NaDES solvent, the following reagents are required: lactic acid, glucose, glycerol, demineralized water, and HBD at a ratio of 1:1, 1:3, 1:5, or 25% w/w. The reagents are then added and mixed using a hotplate stirrer at a temperature of 80°C. The stirring speed should be set to "Number 3" and the mixture should be stirred for up to 3 hours [39–41].

The NaDES extraction process was carried out using a sonicator instrument by designing treatments at temperatures of 30°C, 50°C, and 70°C with durations of 15, 30, and 60 minutes, respectively. The extraction treatments were performed thrice, for a total of 243 extraction treatments. [4,18,33,40,42].

2.5. Identification of Compounds and Characterization of NaDES

The maximum wavelength was ascertained through the utilization of a UV-Vis spectrophotometer, with luteolin serving as the reference standard. The range of wavelengths that yielded maximum absorption was determined to be between 345 and 350 nanometers [43–45] DMSO has been utilized as a solvent for luteolin [46]. Subsequently, a calibration curve was generated by implementing five concentration variations (triplo) utilizing the standard compound luteolin.

The NaDES group has demonstrated considerable aptitude in the development of extraction methodologies. The identification of extracted compounds was subsequently undertaken, employing a technique designated as LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometry). The analysis was performed using mobile phases, namely eluent (A), which consisted of demineralized water and ammonium formate (99.9:0.1), and eluent (B), which consisted of acetonitrile and formic acid (99.9:0.1). The analysis was conducted at a flow rate of 0.2 milliliters per minute, employing a step gradient [4,44–46]. The duration of the procedure was 23 minutes, and the injected volume was 5 µL. A gradient step was performed. The initial interval (0–4 minutes) was allocated exclusively to mobile phase A, while the subsequent interval (4–6 minutes) was devoted to a 40% proportion of mobile phase A. The ensuing interval (16–18 minutes) encompassed 100% of mobile phase B, and the final interval (18–22 minutes) was dedicated entirely to 100% of mobile phase A [22].

CC BY SA

The files with the ".raw" extension were obtained through liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. The Masslynx version 4.1 software was employed to visualize the chromatogram results, which were subsequently converted from the ".raw" extension to ".mzml" using the msconvert software. The (.mzml) file was analyzed using Sirius software, version 6.3.1, to successfully extract the compounds from the NaDES-based analytical method development [4,22].

The PSA instrument is utilized to examine the dimensional characteristics of particles, employing a 25-milliliter sample [8]. The subsequent section is devoted to the presentation of the results obtained from the NaDES extraction process. The Zeta Potential of the system was measured to predict the stability of the NaDES extraction results with zeta values. The zeta potential was measured using Zeta Sizer, which is based on the effective electric charge on the particle surface.

3. RESULT AND DISCUSSION

The focus of this study is the compound known as luteolin; consequently, the identification and characterization of this compound are of paramount importance. The highest detection of luteolin was obtained in the NaDES (natural deep eutectic solvent) extract treatment with choline chloride: lactic acid (1:3) in the ultrasonic treatment for 60 minutes at a temperature of 70º C, yielding a total of 1.2468% (**Fig. 1a**). The NaDES ultrasonic treatment, administered at a temperature of 70º C for a duration of 60 min, was identified as the optimal treatment for achieving the maximum yield percentage of luteolin compound (**Fig. 1b-1d**).

The optimization of temperature in ultrasonic-assisted extraction using NaDES has been demonstrated to yield an optimal yield profile. It has been demonstrated that an increase in temperature from 30 to 70°C results in a substantial enhancement of the percentage yield of the extracted compound. However, temperatures that exceed 70°C lead to a decline in yield, attributable to the thermal degradation of thermolabile compounds [47-49]. The enhancement in ultrasonic-assisted extraction efficiency using natural deep eutectic solvents (NaDES) at elevated temperatures is facilitated by three primary synergistic mechanisms: The reduction in NaDES viscosity has been shown to intensify the mass transfer coefficient and accelerate the rate of molecular diffusion (1). Furthermore, the elevation of molecular kinetic energy has been demonstrated to increase the solubility and mobility of target analytes in the solvent phase (2). Finally, the destabilization of intermolecular interactions between bioactive compounds and plant matrix structural components has been observed, primarily through disruption of cell walls and weakening of hydrogen bonds, which facilitates the release of compounds from cellular compartments (3) [50-52]. An increase in temperature during the extraction process has been shown to result in a decrease in surface tension, thereby enhancing the solvent's capacity to penetrate the porous matrix [53]. The optimization of acoustic cavitation phenomena is achieved through the modulation of thermodynamic parameters. This modulation intensifies the mechanical and chemical effects at optimal operating temperatures [54]. Temperature has been demonstrated to exert a significant influence on the percentage yield of the target compound. However, elevated temperatures, which may be attained during the extraction process, have been observed to exert a deleterious effect on the yield of the target compound. This may also have ramifications for the outcomes of subsequent testing in bioactivity evaluations in silico, in vitro, and in vivo.

The optimization of extraction duration has been shown to induce an increase in % yield through a series of synergistic mechanisms. These mechanisms include (1) intensification of interfacial contact between the solvent phase and the cellular matrix, which accelerates the molecular diffusion of target compounds, (2) achievement of thermodynamic equilibrium in extraction, which requires a characteristic time to optimize the mass transfer rate, and (3) accumulation of acoustic cavitation effects, which results in progressive structural disruption in the ultrasonic-assisted extraction system. However, it has been demonstrated that extending the duration of extraction beyond optimal conditions can induce thermochemical degradation of thermolabile compounds, including flavonoids. In addition, sonochemical degradation of polyphenols can occur through a series of reactions involving free radicals, oxidation, and molecular fragmentation catalysed by cavitation phenomena [55-58]. It has been demonstrated that an increase in the duration of ultrasonic extraction results in an enhancement of the percentage yield of the extracted compound. However, it should be noted that this increase is inherently constrained by a maximum limit. Degradation may potentially occur if extraction is carried out beyond the optimum time.

The characterization of compounds extracted from celery (*Apium graveolens*) by NaDES was performed LC-MS/MS. The results of the LC-MS/MS analysis of the target extraction sample were analyzed with chromatogram analysis with Masslynx software. The ".raw" data from LC-MS/MS analysis was then converted to "MZML" with MSConvert software. The "MZML" data is analyzed with Sirius software to identify the extracted compounds. The luteolin calibration curve by UV-Vis Spectrophotometer was shown in **Fig. 2a**.

A total of thirteen secondary metabolite compounds/NPCs (natural product compounds) were obtained, including ten alkaloids, one terpenoid, and two shikimates and phenylpropanoids (**Fig. 2b**, **Table 1**). The compound luteolin 3'-methyl ether 7-malonylglucoside was successfully extracted in the choline chloride–lactic acid group. A total of fourteen secondary metabolites were obtained, including seven alkaloids, seven shikimates and phenylpropanoids (**Fig. 2c, Table 2**). However, the luteolin compound (Luteolin 7-(6''-malonylneohesperidoside) was second detected in

CC BY SA

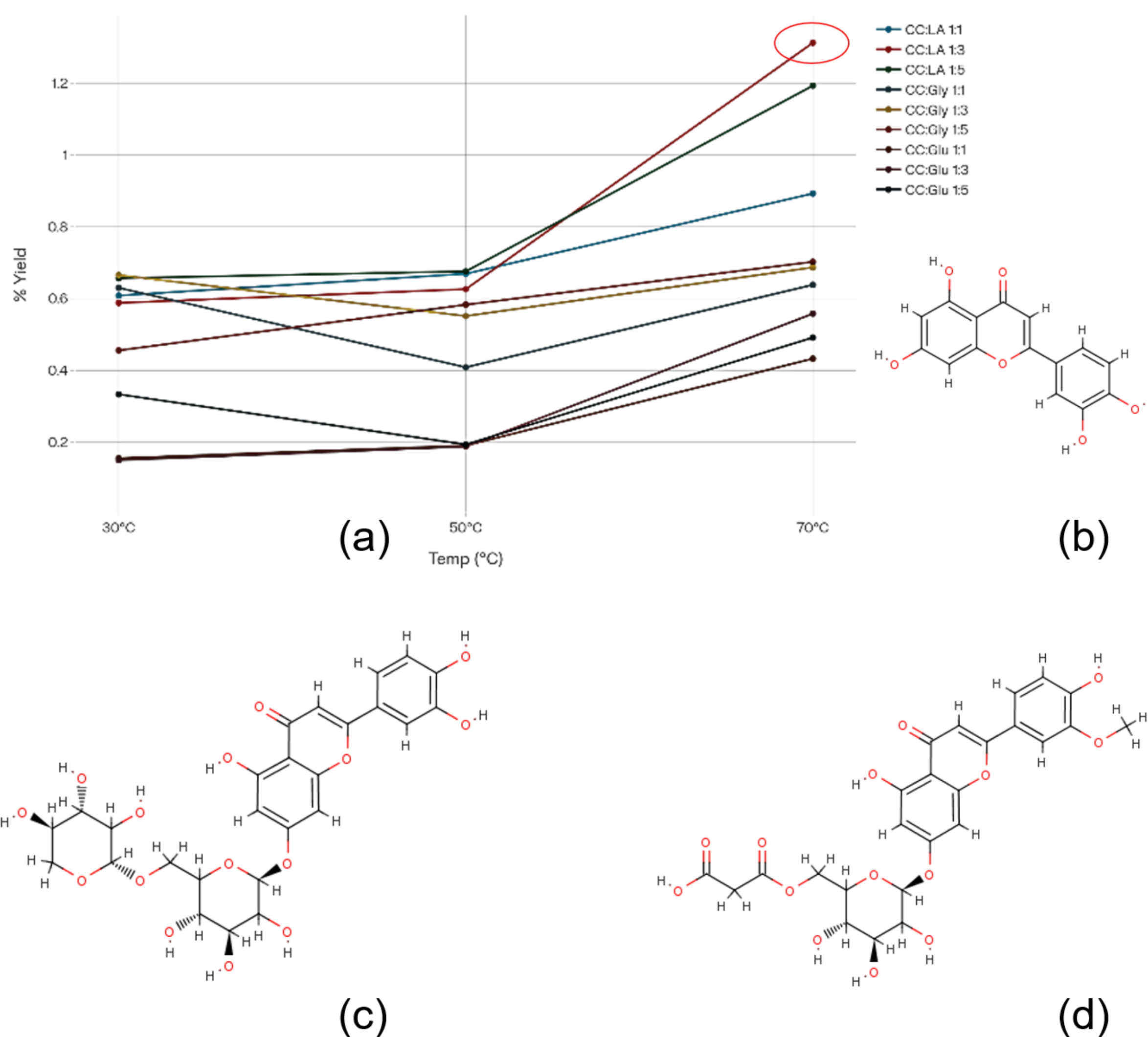

Fig. 2 (a) The % yield of luteolin obtained from NaDES extract of Celery (*Apium graveolens*); Chemical Structure of Luteolin (b), Luteolin 3'-methyl ether 7-malonylglucoside (c), Luteolin 7-Primeveroside (d)

sirius software for the NaDES choline chloride-glycerol group. A total of ten secondary metabolites were obtained, including one alkaloid, three terpenoids, and six shikimates and phenylpropanoids (**Fig. 2c, Table 3**). The extraction of the compound luteolin 7-primeveroside was achieved in the choline chloride–glucose group. These results confirm the presence of luteolin, the target compound for extraction, in celery (*Apium graveolens*).

The choline chloride–lactic acid extract group has been demonstrated to possess a high degree of efficacy in the extraction of complex flavonoid glycoside compounds. The efficacy of choline chloride–lactic acid (ChCl-LA) extraction is contingent upon its high acidity characteristics, with lactic acid functioning as a hydrogen bond donor that provides multiple binding sites through its carboxyl (-COOH) and hydroxyl (-OH) groups.

A comparison of the extraction efficiency of organic acid-based DES (deep eutectic solvent) and polyol-based DES (glycerol) reveals a notable disparity in their respective capabilities. The former exhibits a superior extraction efficiency for polar compounds, such as polyphenols, when compared to the latter. This distinction can be attributed, at least in part, to the higher polarity and lower pH values of the carboxylic acid system associated with polyol-based DES. The molecular structure of lactic acid enables the formation of extensive hydrogen bonding networks with target molecules, particularly glycoside compounds. This process occurs through conformational stabilization and solubility enhancement in polar-acidic media [59-61].

The efficiency of luteolin compound extraction in ChCl-glycerol NaDES serves as an indication of the complexity of molecular interactions in deep eutectic solvents [62]. The phenomenon of extraction failure can be attributed to the incompatibility between the parameters of the target compound, luteolin, which is a moderately polar flavonoid, and the solvation environment characteristics of ChCl-glycerol [63]. The extraction of the pain compound was achieved in this group, thereby demonstrating the efficacy of the treatment in specific groups [64]. Natural compounds containing flavonoid metabolites necessitate solvents that exhibit optimal non-covalent interaction properties.

CC BY SA

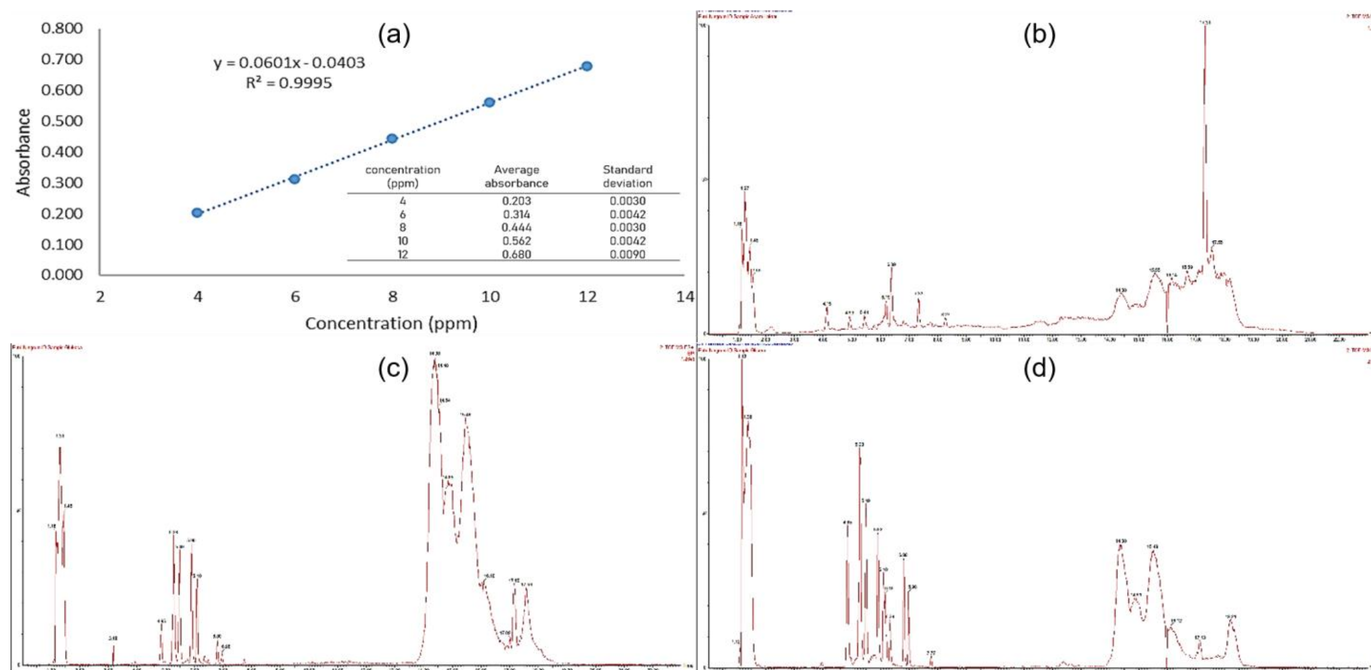

Fig. 2 Luteolin calibration curve (a), Chromatogram NaDES Extract: choline chloride–lactic acid (Luteolin 3'-Methyl Ether 7-Malonylglucoside: RT: 4.920 min.) (b), choline chloride–glycerol (c), choline chloride–glucose (Luteolin 7-Primeveroside: RT: 4.853 min) (d)

Table 1. Characterization chemical compounds in NaDES extract choline chloride – lactic acid by LC-MS-MS

No	Name	Formula	Mass	RT	Confidence Score Exact	NPC pathway
1.	Cordycepin	$C_{10}H_{13}N_5O_3$	251.24	2.861	0.086	Alkaloids
2.	5-Fluoro-4-nitro-1-pentylpyrazol-3-amine	$C_8H_{13}FN_4O_2$	216.21	4.134	0.003	Alkaloids
3.	Pentadecyl nitrite	$C_{15}H_{31}NO_2$	257.41	4.640	0.039	Alkaloids
4.	3-[4-[[2-chloro-5-[(2S,3S,4R,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]phenyl]methyl]phenyl]imidazolidine-2,4-dione	$C_{22}H_{23}ClN_2O_7$	462.90	4.707	0.126	Alkaloids
5.	3-oxo-3-[(3,4,5-trihydroxy-6-{[5-hydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-7-yl]oxy}oxan-2-yl)methoxy]propanoic acid	$C_{24}H_{22}O_{13}$	518.42	4.773	0.861	Shikimates and Phenylpropanoids
6.	**Luteolin 3'-Methyl Ether 7-Malonylglucoside**	$\mathbf{C_{25}H_{24}O_{14}}$	**548.40**	**4.920**	**0.79**	**Shikimates and Phenylpropanoids**
7.	N-ethyl-2-[[2-[2,4,6-trioxo-3,5-bis(prop-2-enyl)-1,3,5-triazinan-1-yl]acetyl]amino]acetamide	$C_{15}H_{21}N_5O_5$	351.36	5.713	0.003	Alkaloids
8.	[2-(4-Ethylanilino)-2-oxoethyl]-methyl-[2-oxo-2-[4-(trifluoromethoxy)anilino]ethyl]azanium	$C_{20}H_{23}F_3N_3O_3$	410.40	5.780	0.008	Alkaloids
9.	1-(4-ethylcyclohexyl)-N-(oxan-4-ylmethyl)methanamine	$C_{15}H_{29}NO$	239.40	5.980	0.037	Terpenoids
10.	N-(4-amino-1,1-difluoro-2-methylbutan-2-yl)-2-methylpropanamide	$C_9H_{18}F_2N_2O$	208.25	6.553	0.007	Alkaloids
11.	4-[4-[Bis(2-ethylhexyl)amino]-2-methylphenyl]imino-3-(2-ethylhexyl)-1,2-oxazol-5-one	$C_{34}H_{57}N_3O_2$	539.80	7.672	0.01	Alkaloids
12.	2,6,6,9,9,13,13,15-Octamethyl-7-(2,2,6,6-tetramethyloctyl)hexadecan-2-amine	$C_{36}H_{75}N$	522.00	7.732	0.127	Alkaloids
13.	1-Tert-butyl-3-(2,2-difluorobutyl)urea	$C_9H_{18}F_2N_2O$	208.25	8.292	0.017	Alkaloids

CC BY SA

Table 2. Characterization chemical compounds in NaDES extract choline chloride – glicerol by LC-MS-MS

No	Name	Formula	Mass	RT	Confidence Score Exact	NPC partway
1.	(2R)-1-(ethylamino)propan-2-ol	$C_5H_{13}NO$	103.100	4.627	0.068	Shikimates and Phenylpropanoids
2.	6''-Malonylapiin	$C_{29}H_{30}O_{17}$	650.500	5.910	0.582	Shikimates and Phenylpropanoids
3.	21-[(2,5-Dioxopyrrol-1-yl)methyl]-28-[(1-ethylcyclopentyl)methylidene]-9-hydroxy-8-methyl-4,29-dioxa-10,11-dithia-17,35-diazaoctacyclo[21.9.2.112,16.01,5.02,22.015,20.024,32.027,31]pentatriaconta-2(22),5,13,15(20),16,18,27(31)-heptaene-3,30-dione	$C_{43}H_{45}N_3O_7S_2$	779.27	4.820	0.014	Shikimates and Phenylpropanoids
4.	N-[4-(1-azepanylsulfonyl)phenyl]-3,5-dinitrobenzamide	$C_{19}H_{20}N_4O_7S$	448.105	4.973	0.001	Shikimates and Phenylpropanoids
5.	Apiin	$C_{26}H_{28}O_{14}$	564.148	5.273	0.690	Shikimates and Phenylpropanoids
6.	5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2S,3R,4S,5S)-3,4,5-trihydroxyoxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	$C_{27}H_{30}O_{15}$	594.158	5.500	0.651	Shikimates and Phenylpropanoids
7.	**3-[[5-[3,4-Dihydroxy-4-(hydroxymethyl)oxolan-2-yl]oxy-3,4-dihydroxy-6-[5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-4-oxochromen-7-yl]oxyoxan-2-yl]methoxy]-3-oxopropanoic acid**	$C_{30}H_{32}O_{18}$	**680.600**	**6.110**	**0.529**	Shikimates and Phenylpropanoids
8.	Apigenin 7-(6'''-Acetylallosyl-(1->2)Glucoside)	$C_{29}H_{32}O_{16}$	636.169	6.973	0.566	Alkaloids
9.	6-ethyl-4-(2-propan-2-ylidenehydrazinyl)-1H-pyridin-2-one	$C_{10}H_{15}N_3O$	193.122	10.331	0.003	Alkaloids
10.	3a,6a-Bis[(phenylmethylidene)amino]-1,3,4,6-tetrakis(allyl)-tetrahydroimidazo[4,5-d]-imidazoline-2,5-dione	$C_{30}H_{32}N_6O_2$	508.259	11.231	0.013	Alkaloids
11.	Ac-DL-Tyr-DL-hArg-DL-Tyr-NH2	$C_{27}H_{37}N_7O_6$	555.281	13.557	0.008	Alkaloids
12.	tert-butyl N-[3-hydroxy-5-[(2-methylpropan-2-yl)oxycarbonylamino]pentyl]carbamate	$C_{15}H_{30}N_2O_5$	318.215	13.630	0.071	Alkaloids
13.	7alpha-(6-Aminohex-1-yl)-bis(tert.-butyldimethylsilanyloxy)estra-1,3,5(10)-triene	$C_{36}H_{65}NO_2Si_2$	599.455	14.063	0.021	Alkaloids
14.	N-[3-(butanoylamino)-5-[(4-tert-butylcyclohexanecarbonyl)amino]-4,4-dimethylcyclohexa-1,5-dien-1-yl]-4-tert-butylcyclohexane-1-carboxamide	$C_{34}H_{57}N_3O_3$	555.440	14.370	0.033	Alkaloids

However, the ChCl-glycerol system is incapable of providing a suitable solvation environment for the effective solubilization of luteolin.

NaDES ChCl-Glucose effectively extracted the compound luteolin 7-primeveroside, which is a glycoside with a complex sugar structure. The thermodynamic stability of the ChCl-glucose system enables the preservation of the primeveroside structure [65]. ChCl-glucose, when combined with water, exhibits moderate effectiveness in the extraction of compounds. This combination can be considered comparable to the effectiveness of methanol in the extraction of flavonoid glycosides [66,67]. The selectivity of flavonoid glycoside extraction with choline chloride-glucose NaDES is related to the principle of structural compatibility. Glucose, acting as a hydrogen bond donor (HBD), possesses multiple hydroxyl groups, which facilitate the

CC BY SA

Table 3. Characterization chemical compounds in NaDES extract choline chloride – glucose by LC-MS-MS

No	Name	Formula	Mass	RT	Confidence Score Exact	NPC pathway
1.	2-Fluorodecane-1,1,1-triol	$C_{10}H_{21}FO_3$	208.147	4.487	0.010	Shikimates and Phenylpropanoids
2.	(2R)-4-(methylamino)butan-2-ol	$C_5H_{13}NO$	103.100	4.554	0.021	Shikimates and Phenylpropanoids
3.	**Luteolin 7-Primeveroside**	$\mathbf{C_{26}H_{28}O_{15}}$	**580.143**	**4.853**	**0.692**	**Shikimates and Phenylpropanoids**
4.	5-aminopentan-1-ol	$C_5H_{13}NO$	103.100	5.193	0.215	Shikimates and Phenylpropanoids
5.	5-hydroxy-2-(4-hydroxy-3-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2S,3R,4S,5S)-3,4,5-trihydroxyoxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	$C_{29}H_{30}O_{18}$	666.143	5.253	0.147	Shikimates and Phenylpropanoids
6.	Apiin	$C_{26}H_{28}O_{14}$	564.148	5.273	0.685	Terpenoids
7.	Ambocin	$C_{26}H_{28}O_{14}$	564.148	5.307	0.656	Terpenoids
8.	3,4,5-trihydroxy-6-((5-hydroxy-6-methoxy-2-(4-methoxyphenyl)-4-oxo-4h-chromen-7-yl)oxy)-N-(2-methoxyphenyl)tetrahydro-2h-pyran-2-carboxamide	$C_{27}H_{30}O_{15}$	594.500	5.500	0.650	Shikimates and Phenylpropanoids
9.	N'-(2-hydroxypropyl)octadecanediamide	$C_{21}H_{42}N_2O_3$	370.320	11.384	0.078	Alkaloids
10.	(2R)-N-[4-oxo-4-[[4-oxo-4-[(2S,4R)-4-propan-2-yloxy-2-(propan-2-yloxymethyl)pyrrolidin-1-yl]butyl]amino]butyl]-2,4-bis(propan-2-ylamino)butanamide	$C_{29}H_{57}N_5O_5$	555.436	14.237	0.017	Terpenoids

formation of extensive hydrogen bonding networks with sugar moieties in target glycoside compounds.

The mean particle size values obtained for each group of choline chloride-lactic acid, choline chloride-glycerol, and choline chloride-glucose were 292.2, 428, and 198.3 nm, respectively (**Fig. 3**), while the poly dispersion index (PDI) values were 0.0284, 0.1404, and 3.7300, respectively (**Table 4**). In contrast, less consistent values were observed in the choline chloride–glycerol and choline chloride–glucose groups. The present study is related to the solubility of the NaDES extract produced in this research.

ChCl-LA displays a monomodal distribution, with a single particle size of 292.2 nm and a PDI of 0.0284. The PDI value in question is notably low, which is indicative of a system that is monodisperse in nature and exhibits a highly uniform particle size distribution [68], [69]. The enhanced homogeneity of the choline chloride-lactic acid system can be ascribed to the inherent structural characteristics of lactic acid, which possesses a single functional carboxyl group and a methyl group. It has been demonstrated that low viscosity values can increase both mass transfer and uniform particle distribution [60,70].

ChCl-Glycerol NaDES manifest a two-population particle distribution: 2197 nm (6%) and 428 nm (94%), accompanied by a PDI of 0.1404, suggesting a moderately polydisperse system. The presence of the minor 2197 nm population suggests the existence of relatively large aggregates in ChCl-Glycerol NaDES [68]. The aggregation phenomenon in ChCl-Glycerol NaDES can be attributed to the elevated viscosity value of glycerol [71]. In this scenario, the addition of water may prove beneficial; however, it is imperative to exercise caution and take into account the bonds that have been established between the NaDES [72]. Glycerol-based N-acetyl-d-glucosamine NaDES have been observed to demonstrate intricate interactions with phospholipid membranes. These interactions have the potential to influence the size distribution of particles within the membrane, which can be achieved through membrane disruption and subsequent reorganization or reassembly [73].

In the ChCl-Glucose NaDES group, this signifies a considerable aggregation [68,69]. The presence of particles measuring 1 nm and 6000 nm is readily apparent in these conditions, where insolubility leads to aggregation. This aggregation can be mitigated by increasing solubility, thereby facilitating a more uniform distribution of particles [64,67]. In certain conditions, DES (deep eutectic solvent) has been observed to undergo nanosegregation, resulting in the formation of separate phases for its individual components [74]. The distribution of particle size has been demonstrated to have substantial implications for the solubility of NaDES extracts. It has been demonstrated that this will have a significant impact on the dissolution rate and bioavailability of bioactive compounds [68,75].

CC BY SA

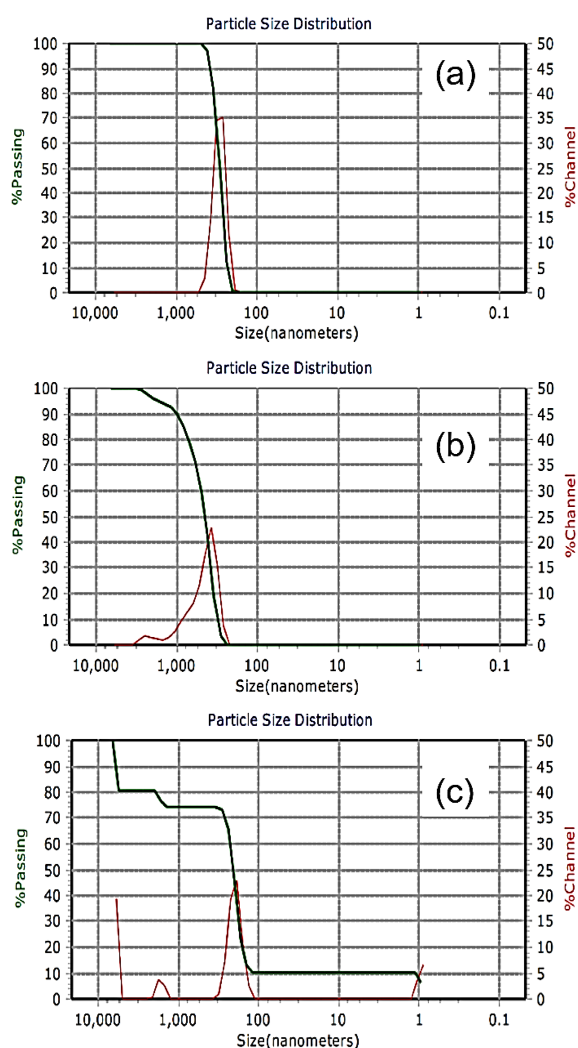

Fig. 3 Particle size distribution of NaDES extract (a) choline chloride-lactic acid, (b) choline chloride-glycerol, and (c) choline chloride-glucose

Table 6. Characterization particle size and zeta potential of NaDES extract of Celery (*Apium graveolens*)

NaDES (HBA–HBD)	Particle size			Zeta potential (mV)
	Diameter (nm)	Vol %	PDI	
Choline chloride–lactic acid	292.2	100.0	0.0284	133.9
Choline chloride–glycerol	2197.0 428.0	6.0 94.0	0.1404	167.0
Choline chloride–glucose	6000.0 1685.0 198.3 1.0	19.5 6.5 70.2 3.8	3.7300	71.0

The zeta potential values for the three NaDES systems demonstrate significant variation and remarkably high values. The following values were obtained: ChCl-LA (133.9 mV), ChCl-Glycerol (167 mV), and ChCl-Glucose (71 mV). The values in question exceed the conventional threshold for colloidal stability, which is generally set at ±30 mV [76,77]. An excessively elevated zeta potential value may serve as an indication of instrument limitations. The findings of this study suggest that the zeta potential values determined for highly conductive electrolyte solutions may not always be an accurate reflection of the electrolyte's true electrokinetic properties [78].

4. CONCLUSION

The optimal %yield of 1.2468% was achieved through choline chloride-lactic acid (1:3) at 60 minutes and a temperature of 70°C. The characterization of luteolin 3'-methyl ether 7-malonylglucoside and luteolin 7-primeveroside compounds was successfully identified in apium graveolens NaDES extract. Characterization of the particle size and zeta potential values of each group of choline chloride–lactic acid, choline chloride–glycerol, and choline chloride–glucose were 292.2 nm, 428 nm, and 198.3 nm, respectively, with corresponding potential values of 167 mV, 133.9 mV, and 71 mV. Celery extract considerable potential for further development. Obtaining samples is a simple process, and the extract exhibits significant promise in supporting Indonesia's autonomy for domain of medicinal raw materials.

5. AUTHOR'S DECLARATION

5.1. Supporting Information

There is no supporting information of this paper. The data that support the findings of this research are available on request from the corresponding author.

5.2. Acknowledgements

We would like to thank KEMENDIKTISAINTEK Republik Indonesia for the support of research funding with the scheme Penelitian Dosen Pemula (PDP) year 2025 with contract number 044/LL10/DT.05.00/PL/2025 and 266/STIKES/JBI/VI/KT-2025.

5.3. Conflict of Interest

There was no conflict of interest in this study.

5.4. Author Contributions

RYP is responsible for research designs, analyzing results, reports, solvent synthesis NaDES, NaDES extraction, analysis of chemical compound detection from NaDES extracts, writing and revising the article. RPM is responsible for analyzing the particle size of NaDES extracts, analyzing the dispersion stability of the best NaDES extract results with potential zeta, percent transmitter and data processing. PNNL is responsible for analyzing luteolin compound levels using UV-Vis Spectrophotometer instruments.

5.5. AI Statement

ChatGPT was utilize to enhance the clarity, grammar, and overall readability of this manuscript. All technical

CC BY SA

content, data interpretation, and conclusion were solely developed and verified by the authors. The final version of the manuscript was thoroughly reviewed to ensure accuracy, coherence, and aligment with the study's findings.

6. REFERENCES

[1] Oomen, W.W., Begines, P., Mustafa, N.R., Wilson, E.G., Verpoorte, R., & Choi, Y.H. 2020. Natural deep eutectic solvent extraction of flavonoids of scutellaria baicalensis as a replacement for conventional organic solvents. *Molecules*. 25(3). 1–11. doi: https://doi.org/10.3390/molecules25030617

[2] Syarifah, A.N., Suryadi, H., Hayun, H., Simamora, A., & Mun'im, A. 2023. Detoxification of comfrey (*Symphytum officinale L.*) extract using natural deep eutectic solvent (NADES) and evaluation of its anti-inflammatory, antioxidant, and hepatoprotective properties. *Front. Pharmacol.* 14. 1–13. doi: https://doi.org/10.3389/fphar.2023.1012716

[3] Yang, G.Y., Song, J.N., Chang, Y.Q., Wang, L., Zheng, Y.G., Zhang, D., & Guo, L. 2021. Natural deep eutectic solvents for the extraction of bioactive steroidal saponins from dioscoreae nipponicae rhizoma. *Molecules*. 26(7). 1–14. doi: https://doi.org/10.3390/molecules26072079

[4] Putra, R.Y., Mariska, R.P., & Latifah, P.N.N. 2024. Ultrasonic NaDES-based optimization of luteolin extraction technology from celery (Apium graveolens) for improved drug raw material independence. *Ris. Inf. Kesehat.* 13(2). 134–143. doi: https://doi.org/10.30644/rik.v13i2.906

[5] Yulion, R., Aliyah, S.H., Perawati, S., Andriani, L., & Eryunita, P. 2024. Novel Natural Deep Eutectic Solvent (NaDES) yellow choline chloride and molecular docking soybean extract (*Glycine max*) as diabetes drugs candidate. *Indones. J. Chem. Stud.* 3(2). 46–51. doi: https://doi.org/10.55749/ijcs.v3i2.47

[6] Budhy, T.I., Adam, D., Azis, Z.M.R., Syahputri, V., Yuliani, M.G.A., Suwarto, M.F.S., & Setiawan, F. 2024. The potential of moringa leaf nanoparticles (*Moringa oleifera*) on the expression of TNFα, IL10, and HSP 27 in oral cavity cancer. *J. Multidiscip. Appl. Nat. Sci.* 4(1). 120–129. doi: https://doi.org/10.47352/jmans.2774-3047.198

[7] Kuru, C.İ., Ulucan-Karnak, F., Dayıoğlu, B., Şahinler, M., Şendemir, A., & Akgöl, S. 2024. Affinity-based magnetic nanoparticle development for cancer stem cell isolation. *Polymers (Basel).* 16(2). doi: https://doi.org/10.3390/polym16020196

[8] Haflin, Agusriani, Mustofa, K., Mariska, R.P., Yulion, R., Pitriani, Al Fadhilah, S., & Hartesi, B. 2024. Formulation and immunomodulatory bioactivity test of nanoparticle syrup of ethanol extract of sungkai leaves (*Peronema canescens Jack*). *Med. Sains J. Ilm. Kefarmasian*. 9(1). 253–270. doi: https://doi.org/10.37874/ms.v9i1.936

[9] Sapone, V., Cicci, A., Franceschi, D., Vincenzi, S., & Bravi, M. 2020. Antioxidant extraction and bioactivity preservation from winery by-products by natural deep eutectic solvents (NaDES). *Chem. Eng. Trans.* 79. 157–162. doi: https://doi.org/10.3303/CET2079027

[10] Aduloju, E.I., Yahaya, N., Mohammad Zain, N., Anuar Kamaruddin, M., & Ariffuddin Abd Hamid, M. 2023. An overview on the use of deep eutectic solvents for green extraction of some selected bioactive compounds from natural matrices. *Adv. J. Chem. Sect. A.* 6(3). 253–300. doi: https://doi.org/10.22034/ajca.2023.389403.1356

[11] Benvenuto, M.A. & Welch, L.A. 2023. *Green Chemistry Processing*, 10th ed. Berlin/Boston: Walter de Gruyter GmbH.

[12] Grudniewska, A. & Pastyrczyk, N. 2022. New insight for spent hops utilization: simultaneous extraction of protein and xanthohumol using deep eutectic solvents. *Biomass Convers. Biorefinery*. 0123456789. doi: https://doi.org/10.1007/s13399-022-03462-5

[13] García-Roldán, A., Piriou, L., & Jauregi, P. 2023. Natural deep eutectic solvents as a green extraction of polyphenols from spent coffee ground with enhanced bioactivities. *Front. Plant Sci.* 13. 1–11. doi: https://doi.org/10.3389/fpls.2022.1072592

[14] Maimulyanti, A., Nurhidayati, I., Mellisani, B., Amelia R.P.F., Puspita, F., & Restu P.A. 2023. Development of natural deep eutectic solvent (NADES) based on choline chloride as a green solvent to extract phenolic compound from coffee husk waste. *Arab. J. Chem.* 16(4). 104634. doi: https://doi.org/10.1016/j.arabjc.2023.104634

[15] Momotko, M., Łuczak, J., Przyjazny, A., & Boczkaj, G. 2022. A natural deep eutectic solvent - protonated L-proline-xylitol - based stationary phase for gas chromatography. *J. Chromatogr. A.* 1676. 463238. doi: https://doi.org/10.1016/j.chroma.2022.463238

[16] Yulion, R., Yulianis, Y., & Suntri, S. 2023. Quercetin Bioavailability Evaluation on Standardized Herbal Medicine Containing Guava Leaf Extract with HPLC. *Biol. Med. Nat. Prod. Chem.* 12(2). 593–599. doi: https://doi.org/10.14421/biomedich.2023.122.593-599

[17] Rivai, H., Yulion Putra, R., & Krisyanella, dan. 2012. Penentuan pengaruh jenis pelarut pengekstrak terhadap perolehan kadar senyawa fenolat dan aktifitas antioksidan dari daun jambu biji (*Psidium guajava L.*). *Jurnal Farmasi Higea*. 4(1). 16-23. doi: http://dx.doi.org/10.52689/higea.v4i1.57

[18] Popovic, B.M., Micic, N., Potkonjak, A., Blagojevic, B., Pavlovic, K., Milanov, D., & Juric, T. 2022. Novel extraction of polyphenols from sour cherry pomace using natural deep eutectic solvents – Ultrafast microwave-assisted NADES preparation and extraction. *Food Chem.* 366. 130562. doi: https://doi.org/10.1016/j.foodchem.2021.130562

[19] Mansur, A.R., Song, N.E., Jang, H.W., Lim, T.G., Yoo, M., & Nam, T.G. 2019. Optimizing the ultrasound-assisted deep eutectic solvent extraction of flavonoids in common buckwheat sprouts. *Food Chem.* 293. 438–445. doi: https://doi.org/10.1016/j.foodchem.2019.05.003

[20] Bertolo, M.R.V., Bogusz Junior, S., & Mitchell, A.E. 2023. Green strategies for recovery of bioactive phenolic compounds from agro-industrial wastes (pomegranate peels, almond hulls, and elderberry pomace) using natural deep eutectic solvents. *ACS Food Sci. Technol.* 3(12). 2144–2156. doi: https://doi.org/10.1021/acsfoodscitech.3c00367

[21] Chanioti, S. & Tzia, C. 2018. Extraction of phenolic compounds from olive pomace by using natural deep eutectic solvents and innovative extraction techniques. *Innov. Food Sci. Emerg. Technol.* 48. 228–239. doi: https://doi.org/10.1016/j.ifset.2018.07.001

[22] Yulion, R., Andriani, L., Asadi, H.A., 'Aliyah, S.H., Mariska, R.P., Perawati, S., & Hasanah, R. 2023. Spray Gel Formulation of Ethanolic Extract Senduduk Leaves (*Melastoma malabatricum*) Against Antioxidant Activity, SPF, and Chemical Components Analysis with LC-MS/MS. *Indones. J. Chem. Stud.* 2(2). 67–75. doi: https://doi.org/10.55749/ijcs.v2i2.37

[23] Hestari, W.A., Mariska, R.P., & Agung, M.H. 2024. Pengaruh alginat dan $CaCl_2$ terhadap formulasi nanopartikel ekstrak etanol daun teh (*Camellia sinensis (L.) Kuntze*) kerinci sebagai antioksidan alami. *J. Pengemb. ILMU Pengetah.* 5(4). 1–16.

[24] Turner, L., Lignou, S., Gawthrop, F., & Wagstaff, C. 2021. Investigating the factors that influence the aroma profile of Apium graveolens: A review. *Food Chem.* 345. 128673. doi: https://doi.org/10.1016/j.foodchem.2020.128673

[25] Hedayati, N., Bemani Naeini, M., Mohammadinejad, A., & Mohajeri, S.A. 2019. Beneficial effects of celery (Apium graveolens) on metabolic syndrome: A review of the existing evidences. *Phyther. Res.* 33(12). 3040–3053. doi: https://doi.org/10.1002/ptr.6492

[26] Kooti, W. & Daraei, N. 2017. A Review of the Antioxidant Activity of Celery (*Apium graveolens L*). *J. Evidence-Based Complement. Altern. Med.* 22(4). 1029–1034. doi: https://doi.org/10.1177/2156587217717415

[27] Lopez-Lazaro, M. 2009. Distribution and Biological Activities of the Flavonoid Luteolin. *Mini-Reviews Med. Chem.* 9(1). 31–59. doi: https://doi.org/10.2174/138955709787001712

[28] Punia Bangar, S., Kajla, P., Chaudhary, V., Sharma, N., & Ozogul, F. 2023. Luteolin: A flavone with myriads of bioactivities and food applications. *Food Biosci.* 52. 102366. doi: https://doi.org/10.1016/j.fbio.2023.102366

[29] Kure, A., Nakagawa, K., Kondo, M., Kato, S., Kimura, F., Watanabe, A., Shoji, N., Hatanaka, S., Tsushida, T. and Miyazawa, T. 2016. Metabolic fate of luteolin in rats: Its relationship to anti-inflammatory effect. *J. Agric. Food Chem.*

CC BY SA

64(21). 4246–4254. doi: https://doi.org/10.1021/acs.jafc.6b00964

[30] Dang, H., Meng, M.H.W., Zhao, H., Iqbal, J., Dai, R., Deng, Y., & Lv, F. 2014. Luteolin-loaded solid lipid nanoparticles synthesis, characterization, & improvement of bioavailability, pharmacokinetics in vitro and vivo studies. *J. Nanoparticle Res.* 16(4). doi: https://doi.org/10.1007/s11051-014-2347-9

[31] Çetinkaya, M. & Baran, Y. 2023. Therapeutic potential of luteolin on cancer. *Vaccines*. 11(3). 554. doi: https://doi.org/10.3390/vaccines11030554

[32] Basha, N.J. & Basavarajaiah, S.M. 2023. Anticancer Potential of Bioactive Molecule Luteolin and Its Analogs: An Update. *Polycycl. Aromat. Compd.* 43(5). 3958–3976. doi: https://doi.org/10.1080/10406638.2022.2080728

[33] Ozkan, G. 2024. Valorization of artichoke outer petals by using ultrasound-assisted extraction and natural deep eutectic solvents (NADES) for the recovery of phenolic compounds. *J. Sci. Food Agric.* 104(5). 2744–2749. doi: https://doi.org/10.1002/jsfa.13158

[34] Juszczak, A.M., Marijan, M., Jakupović, L., Tomczykowa, M., Tomczyk, M., & Zovko Končić, M. 2023. Glycerol and natural deep eutectic solvents extraction for preparation of luteolin-rich jasione montana extracts with cosmeceutical activity. *Metabolites*. 13(1). 32. doi: https://doi.org/10.3390/metabo13010032

[35] Sakti, A.S., Saputri, F.C., & Mun'im, A. 2019. Optimization of choline chloride-glycerol based natural deep eutectic solvent for extraction bioactive substances from *Cinnamomum burmannii* barks and *Caesalpinia sappan* heartwoods. *Heliyon*. 5(12). e02915. doi: https://doi.org/10.1016/j.heliyon.2019.e02915

[36] Zain, M.S.C., Yeoh, J.X., Lee, S.Y., & Shaari, K. 2021. Physicochemical properties of choline chloride-based natural deep eutectic solvents (Nades) and their applicability for extracting oil palm flavonoids. *Sustain.* 13(23). 12981. doi: https://doi.org/10.3390/su132312981

[37] Shang, X., Zhang, M., Hu, J., Zhang, Y., Yang, L., & Hou, X. 2023. Chemical compositions, extraction optimizations, and in vitro bioactivities of flavonoids from perilla leaves (perillae folium) by microwave-assisted natural deep eutectic solvents. *Antioxidants*. 12(1). 104. doi: https://doi.org/10.3390/antiox12010104

[38] Palos-Hernández, A., Gutiérrez Fernández, M.Y., Escuadra Burrieza, J., Pérez-Iglesias, J.L., & González-Paramás, A.M. 2022. Obtaining green extracts rich in phenolic compounds from underexploited food by-products using natural deep eutectic solvents. Opportunities and challenges. *Sustain. Chem. Pharm.* 29. 100773. doi: https://doi.org/10.1016/j.scp.2022.100773

[39] Teslić, N., Santos, F., Oliveira, F., Stupar, A., Pojić, M., Mandić, A., Pavlić, B., Kljakić, A.C., Duarte, A.R.C., Paiva, A. and Mišan, A. 2022. Simultaneous hydrolysis of ellagitannins and extraction of ellagic acid from defatted raspberry seeds using natural deep eutectic solvents (NADES). *Antioxidants*. 11(2). 254. doi: https://doi.org/10.3390/antiox11020254

[40] Aryati, W.D., Nadhira, A., Febianli, D., Fransisca, F., & Mun'im, A. 2020. Natural deep eutectic solvents ultrasound-assisted extraction (Nades-uae) of trans-cinnamaldehyde and coumarin from cinnamon bark [cinnamomum burmannii (nees & t. nees) blume]. *J. Res. Pharm.* 24(3). 389–398. doi: https://doi.org/10.35333/jrp.2020.161

[41] Zurob, E., Cabezas, R., Villarroel, E., Rosas, N., Merlet, G., Quijada-Maldonado, E., Romero, J., & Plaza, A. 2020. Design of natural deep eutectic solvents for the ultrasound-assisted extraction of hydroxytyrosol from olive leaves supported by COSMO-RS. *Sep. Purif. Technol.* 248. 117054. doi: https://doi.org/10.1016/j.seppur.2020.117054

[42] Ahmad, I., Syakfanaya, A.M., Azminah, A., Saputri, F.C., & Mun'im, A. 2021. Optimization of betaine-sorbitol natural deep eutectic solvent-based ultrasound-assisted extraction and pancreatic lipase inhibitory activity of chlorogenic acid and caffeine content from robusta green coffee beans. *Heliyon*. 7(8). e07702. doi: https://doi.org/10.1016/j.heliyon.2021.e07702

[43] Gupta, A., Behl, T., Singh, S., Garg, M., Tamboli, E.T., Chigurupati, S., Felemban, S.G., Albarrati, A., Albratty, M. and Meraya, A.M. 2023. Quantification of Luteolin, Apigenin and Chrysoeriol in Tecoma stans by RP-HPLC Method. *J. Chromatogr. Sci.* 61(9). 844–851. doi: https://doi.org/10.1093/chromsci/bmad022

[44] Xiaoxv Dong, Lan, W., Yin, X., Yang, C., Wang, W., & Ni, J. 2018. Simultaneous determination and pharmacokinetic study of quercetin, luteolin, and apigenin in rat plasma after oral administration of *Matricaria chamomilla L.* extract by HPLC-UV. *Biomed. Chromatogr.* 32(3). 1–8. doi: https://doi.org/10.1002/bmc.4129

[45] Sevindik, H.G., Ozgen, U., Atila, A., Ozturk Er, H., Kazaz, C., & Duman, H. 2015. Phtytochemical studies and quantitative hplc analysis of rosmarinic acid and luteolin 5-*O*-β-D-glucopyranoside on *Thymus praecox* subsp. *grossheimii* var. *grossheimii*. *Chem. Pharm. Bull. (Tokyo).* 63(9). 720–725. doi: https://doi.org/10.1248/cpb.c14-00877

[46] Rajhard, S., Hladnik, L., Vicente, F.A., Srčič, S., Grilc, M., & Likozar, B. 2021. Solubility of luteolin and other polyphenolic compounds in water, nonpolar, polar aprotic and protic solvents by applying ftir/hplc. *Processes*. 9(11). 1952. doi: https://doi.org/10.3390/pr9111952

[47] Xu, Z., Da, X., Qu, J., & Xiao, S. 2024. Natural deep eutectic solvent-based ultrasound-assisted extraction of flavonoids from *Fagopyrum tataricum* bran. *Separations*. 11(5). 1–16. doi: https://doi.org/10.3390/separations11050145

[48] Vo, T.P., Pham, T.V., Weina, K., Tran, T.N.H., Vo, L.T.V., Nguyen, P.T., Bui, T.L.H., Phan, T.H., & Nguyen, D.Q. 2023. Green extraction of phenolics and flavonoids from black mulberry fruit using natural deep eutectic solvents: optimization and surface morphology. *BMC Chem.* 17(1). 1–15. doi: https://doi.org/10.1186/s13065-023-01041-x

[49] Du, G., Hong, W., Li, Z., Liu, Y., & Wang, C. 2023. Process optimization of deep eutectic solvent-based microwave-assisted extraction of flavonoids from Ziziphi Spinosae Semen using response surface methodology. *Food Sci. Technol.* 43. 1–10. doi: https://doi.org/10.1590/fst.122622

[50] Peng, Z., Wang, Y., Li, W., Zhan, B., Zhu, L., Yang, D., Li, G., Zhang, L., & Zhao, Z. 2025. Ultrasonic-assisted extraction of flavonoids from Amomum villosum Lour. Using natural deep eutectic solvent: Process optimization, comparison, identification, and bioactivity. *Ultrason. Sonochem.* 116. 107304. doi: https://doi.org/10.1016/j.ultsonch.2025.107304

[51] Yan, Y.C., Rashmi, W., Khalid, M., Shahbaz, K., Gupta, T.C.S.M., & Mase, N. 2017. Potential application of deep eutectic solvents in heat transfer application. *J. Eng. Sci. Technol.* 12. 1–14.

[52] Gao, J., Xie, L., Peng, Y., Li, M., Li, J., Ni, Y., & Wen, X. 2024. Deep eutectic solvents as new extraction media for flavonoids in mung bean. *Foods*. 13(5). 1–17. doi: https://doi.org/10.3390/foods13050777

[53] Weldegebrieal, G.K. 2020. Synthesis method, antibacterial and photocatalytic activity of ZnO nanoparticles for azo dyes in wastewater treatment: A review. *Inorg. Chem. Commun.* 120. 108140. doi: https://doi.org/10.1016/j.inoche.2020.108140

[54] Korobiichuk, I., Mel'nick, V., Ostapenko, Z., & Ruzhinska, L. 2023. Study of heat and mass transfer processes during extraction of plant raw materials under the influence of ultrasound. *Ultrason. Sonochem.* 98. 106512. doi: https://doi.org/10.1016/j.ultsonch.2023.106512

[55] Yusnira & Ediputra, K. 2025. Optimization of extraction of curcuminoids from temulawak (*Curcuma xanthorrhiza Roxb.*) using response surface methodology. *J. Penelit. Pendidik. IPA.* 11(8). 183–192. doi: https://doi.org/10.29303/jppipa.v11i8.12108

[56] Hu, D.B., Xue, R., Zhuang, X.C., Zhang, X.S., & Shi, S.L. 2023. Ultrasound-assisted extraction optimization of polyphenols from Boletus bicolor and evaluation of its antioxidant activity. *Front. Nutr.* 10. 1–14. doi: https://doi.org/10.3389/fnut.2023.1135712

[57] Abi-Khattar, A.M., Boussetta, N., Rajha, H.N., Abdel-Massih, R.M., Louka, N., Maroun, R.G., Vorobiev, E., & Debs, E. 2022. Mechanical damage and thermal effect induced by ultrasonic treatment in olive leaf tissue. Impact on polyphenols recovery. *Ultrason. Sonochem.* 82. doi: https://doi.org/10.1016/j.ultsonch.2021.105895

[58] Azahar, N.F., Gani, S.S.A., & Mohd Mokhtar, N.F. 2017. Optimization of phenolics and flavonoids extraction conditions of Curcuma Zedoaria leaves using response surface methodology. *Chem. Cent. J.* 11(1). 1–10. doi: https://doi.org/10.1186/s13065-017-0324-y

[59] Benítez-Correa, E., Bastías-Montes, J.M., Acuña-Nelson, S., & Muñoz-Fariña, O. 2023. Effect of choline chloride-based deep

eutectic solvents on polyphenols extraction from cocoa (*Theobroma cacao L.*) bean shells and antioxidant activity of extracts. *Curr. Res. Food Sci.* 7. 100614. doi: https://doi.org/10.1016/j.crfs.2023.100614

[60] Fanali, C., Gallo, V., Della Posta, S., Dugo, L., Mazzeo, L., Cocchi, M., Piemonte, V. and De Gara, L. 2021. Choline chloride–lactic acid-based NADES as an extraction medium in a response surface methodology-optimized method for the extraction of phenolic compounds from hazelnut skin. *Molecules*. 255(9). 117737. doi: https://doi.org/10.1016/j.seppur.2020.117737

[61] Ivanović, M., Grujić, D., Cerar, J., Razboršek, M.I., Topalić-Trivunović, L., Savić, A., Kočar, D., & Kolar, M. 2022. Extraction of bioactive metabolites from achillea millefolium l. with choline chloride based natural deep eutectic solvents: a study of the antioxidant and antimicrobial activity. *Antioxidants*. 11(4). 714. doi: https://doi.org/10.3390/antiox11040724

[62] Puspita Sari, I., Artiningsih Octaviani, E., Dhiya Khansa, S., & Fadillah, G. 2024. Spectroscopic insights and physicochemical properties of deep eutectic solvents formed from choline chloride and organic hydrogen bond donors. *Jati Agung Dist. South Lampung 35365 Anal. Anal. Environ. Chem.* 9(02). 11–22. doi: http://dx.doi.org/10.23960/analit.v9i02.202Anal.Environ.Chem

[63] Kowalska, G., Baj, T., Kowalski, R., & Szymańska, J. 2021. Optimization of glycerol–water extraction of selected bioactive compounds from peppermint and common nettle. *Antioxidants*. 10(5). doi: https://doi.org/10.3390/antiox10050817

[64] Zain, M.S.C., Yeoh, J.X., Lee, S.Y. and Shaari, K., 2021. Physicochemical properties of choline chloride-based natural deep eutectic solvents (NaDES) and their applicability for extracting oil palm flavonoids. *Sustainability*. 13(23). 12981. doi: https://doi.org/10.3390/antiox10111802

[65] Grozdanova, T., Trusheva, B., Alipieva, K., Popova, M., Dimitrova, L., Najdenski, H., Zaharieva, M.M., Ilieva, Y., Vasileva, B., Miloshev, G. and Georgieva, M., 2020. Extracts of medicinal plants with natural deep eutectic solvents: Enhanced antimicrobial activity and low genotoxicity. *BMC Shem.* 14(1). 73. doi: https://doi.org/10.1186/s13065-020-00726-x

[66] Kovač, M.J., Jokić, S., Jerković, I. and Molnar, M. 2022. Optimization of deep eutectic solvent extraction of phenolic acids and tannins from *Alchemilla vulgaris L. Plants*. 11(4). 474. doi: https://doi.org/10.3390/plants11040474

[67] Taco, V., Almachi, D., Bonilla, P., Gijón-Arreortúa, I., Benali, S., Raquez, J.M., Duez, P., & Nachtergael, A. 2025. Natural Deep Eutectic Solvents (NADESs) for the extraction of bioactive compounds from quinoa (*Chenopodium quinoa Willd.*) leaves: a semi-quantitative analysis using high performance thin-layer chromatography. *Molecules*. 30(12). 2620. doi: https://doi.org/10.3390/molecules30122620

[68] Danaei, M., Dehghankhold, M., Ataei, S., Hasanzadeh Davarani, F., Javanmard, R., Dokhani, A., Khorasani, S., & Mozafari, M.R. 2018. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics*. 10(2). 1–17. doi: https://doi.org/10.3390/pharmaceutics10020057

[69] Nanotempertech.com. 2025. Polydispersity Index (PDI), *nanotempertech.com*.https://support.nanotempertech.com/hc/en-us/articles/23808814599057-Polydispersity-Index-PDI (accessed Sep. 21, 2025).

[70] Al-Risheq, D.I.M., Nasser, M.S., Qiblawey, H., Hussein, I.A., & Benamor, A. 2021. Choline chloride based natural deep eutectic solvent for destabilization and separation of stable colloidal dispersions. *Sep. Purif. Technol.* 255. 117737. doi: https://doi.org/10.1016/j.seppur.2020.117737

[71] Ijardar, S.P., Singh, V., & Gardas, R.L. 2022. Revisiting the physicochemical properties and applications of deep eutectic solvents. *Molecules*. 27(4). 1–37. doi: https://doi.org/10.3390/molecules27041368

[72] Kivelä, H., Salomäki, M., Vainikka, P., Mäkilä, E., Poletti, F., Ruggeri, S., Terzi, F., & Lukkari, J. 2022. Effect of water on a hydrophobic deep eutectic solvent. *J. Phys. Chem. B*. 126(2). 513–527. doi: https://doi.org/10.1021/acs.jpcb.1c08170

[73] Nystedt, H.L., Grønlien, K.G., & Tønnesen, H.H. 2021. Interactions of natural deep eutectic solvents (NADES) with artificial and natural membranes. *J. Mol. Liq.* 328. doi: https://doi.org/10.1016/j.molliq.2021.115452

[74] Komal, Singh, G., Singh, G., & Kang, T.S. 2018. Aggregation behavior of sodium dioctyl sulfosuccinate in deep eutectic solvents and their mixtures with water: an account of solvent's polarity, cohesiveness, and solvent structure. *ACS Omega*. 3(10). 13387–13398. doi: https://doi.org/10.1021/acsomega.8b01637

[75] Varenne, F., Makky, A., Gaucher-Delmas, M., Violleau, F., & Vauthier, C. 2016. Multimodal dispersion of nanoparticles: a comprehensive evaluation of size distribution with 9 size measurement methods. *Pharm. Res.* 33(5). 1220–1234. doi: https://doi.org/10.1007/s11095-016-1867-7

[76] Waters. 2025. Zeta Potential, *Waters*. https://www.wyatt.com/solutions/properties/charge-zeta-potential.html (accessed Sep. 21, 2025).

[77] Németh, Z., Csóka, I., Semnani Jazani, R., Sipos, B., Haspel, H., Kozma, G., Kónya, Z., & Dobó, D.G. 2022. Quality by Design-Driven Zeta Potential Optimisation Study of Liposomes with Charge Imparting Membrane Additives. *Pharmaceutics*. 14(9). doi: https://doi.org/10.3390/pharmaceutics14091798

[78] Kaszuba, M., Corbett, J., Watson, F.M.N., & Jones, A. 2010. High-concentration zeta potential measurements using light-scattering techniques. *Philos. Trans. R. Soc. A Math. Phys. Eng. Sci.* 368(1927). 4439–4451. doi: https://doi.org/10.1098/rsta.2010.0175