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Ultrasonic NaDES-based optimization of luteolin extraction technology from celery (Apium graveolens) for improved drug raw material independence

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Abstract

Background: Natural deep eutectic solvents (NaDES) represent a significant advancement in the field of natural material extraction, offering a novel approach to the isolation of active compounds. The objective of this study was to develop a novel NaDES-based extraction method to achieve the highest percentage of luteolin compounds in celery (Apium graveolens) through ultrasonic treatment.
Method: Choline chloride was employed as the hydrogen bonding acceptor (HBA), while lactic acid and malic acid served as the hydrogen bonding donors (HBD). Treatment optimization was conducted using Design Expert 7 software, with the variables comprising the HBA:HBD ratio, temperature, and time.
Results: The optimal combination of NaDES solvents was identified as choline chloride: lactic acid and choline chloride: malic acid at a ratio of (4.71 mol: 4.76 mol) and (1.27 mol: 1.71 mol), respectively, at a temperature of 55.2°C. The optimal conditions were 40°C and 35.45°C, with a time of 15.63 minutes and 12.73 minutes, respectively, which yielded 2.2789% and 0.0102% with a desirability of 0.2788 and 0.0408, respectively.
Conclusion: The optimal combination was identified as choline chloride: lactic acid, which demonstrated a gain of over 2%. Additionally, the combination of choline chloride: malic acid proved effective for luteolin extraction.

Keywords: Choline Chloride; Green extraction; Lactic acid; Malic acid; NaDES (Natural deep eutectic solvents); Ultrasound-assisted extraction

INTRODUCTION

The food, cosmetic, and pharmaceutical industries continue to utilize organic solvents, also referred to as conventional solvents, in their extraction processes. These solvents include methanol, acetone, benzene, chloroform, petroleum ether, and hexane (1,2). The majority of conventional solvents are flammable, explosive, poorly biodegradable, and possess high toxicity (3). At present, the extraction process of natural materials has been conducted up to the fractionation stage, with further studies underway to assess the suitability of the resulting pharmaceutical dosage forms and to evaluate their toxicity (4–8). The use of natural deep eutectic solvents (NaDES) as an alternative to conventional solvents is a promising avenue of research, particularly in light of the ongoing development of extraction technology.

The potential of NaDES extraction technology lies in its capacity to yield more targeted compound extraction results. The use of eutectic solvents, which are environmentally friendly, non-flammable, non-volatile, effective, efficient, inexpensive, and non-toxic, further enhances this technology (9,10). NADES is the development of an analogous ionic liquid solvent (ILs) better known as DES (Deep Eutectic Solvent). (11). The process entails the mixing of hydrogen bond acceptors (HBA) and hydrogen bond donors (HBD) in a specific molar ratio until the formation of hydrogen bonds is achieved (12–14). The resulting solution is then mixed with water at a specific ratio (volumetric to volumetric). NADES is formed from natural eutectic compounds derived from plant

metabolites. Choline chloride (ChCl), citric acid, malic acid, maleic acid, acetic acid, glucose, fructose, sucrose, trehalose, terpenoids, or water have been used as NADES (15–17). NADES are biodegradable and have minimal toxicity because they are easier to break down in the environment. (18). NADES can function as a natural solvent, as evidenced by their efficacy in natural matrices. (19,20).

The utilization of UV-Vis instruments in the analysis and identification of compounds, as well as the detection of yields, is a crucial aspect of modern chemical research (21,22) The luteolin compound was extracted using a NaDES-based method. Previous studies have demonstrated the efficacy of the ultrasound-assisted extraction (UAE) method with natural deep eutectic solvents (NADES). This method has been shown to increase cell permeability, thereby reducing the time required for extraction and increasing the yield of the process. (23). NADES has been demonstrated to be an effective solvent for ultrasonic-assisted extraction (UAE) of flavonoids and phenolics. Its efficacy in this regard is even superior to that of methanol, a commonly used solvent for the extraction of these compounds (24–26). NADES exhibit a number of beneficial properties, including sustainability, biodegradability, compositional flexibility, and the ability to extract bioactive compounds. These characteristics make NADES an attractive option for use as an environmentally friendly green solvent in the development of extraction methods.

Celery (Apium graveolens) is a vegetable that is consumed with regularity, imparting a robust and distinctive flavor to a variety of dishes while conferring a number of health benefits (27). A compound, luteolin, has been identified in celery. (28–30). Luteolin is a flavonoid secondary metabolite that exhibits antimicrobial bioactivity (31), anti-inflammatory (32), anti-cancer (30,33–35), antioxidant (29) anti-viral (35) anti-diabetic and anti-obesity (28).

The NADES with the hydrogen bonding acceptor (HBA) choline chloride was selected as the most promising option (36). Lactic acid and malic acid were selected as hydrogen bonding donors (HBDs) due to their demonstrated ability to extract compounds with flavonoid secondary metabolites (23,25,37). The advancement of extraction technology from the conceptualization of this methodology is to achieve enhanced yields in celery extraction outcomes with the objective of isolating the target active compound luteolin.

METHOD

Tools and Materials

UV-Vis spectrophotometer (Shimadzu® UV-1800), Kuvet, ultrasonic (BAKU® BK-1200), hotplate steerer (IKA® C-MAG HS7), Erlenmeyer (IWAKI® Pyrex), glass bottles, beakers (IWAKI® Pyrex), centrifuge (Hettich® EBA 20), Whatman filter paper No.1, stirring rods (5 cm), Celery (Apium graveolens), luteolin, choline chloride, lactic acid, malic acid, DMSO, aqua demineralized.

Procedure

The sampling was conducted in Jambi City, Jambi Province. The celery (Apium graveolens) leaf samples were used in this study. The determination was carried out at the biology laboratory of Gadjah Mada University (UGM).

NaDES (Natural Deep Eutectic Solvent) Preparation

The sampling was conducted in Jambi City, Jambi Province. The celery (Apium graveolens) leaf samples were used in this study. The determination was carried out at the biology laboratory of Gadjah Mada University (UGM) (23,37–39). The NaDES component was weighed in accordance with the predetermined ratio and demineralized water was added. The mixture was then homogenized using a hotplate steamer at 80°C for up to one hour. The rotation speed was set at "no. 3" until a clear NaDES solvent was obtained (17,36,40,41).

NaDES extraction method (Natural Deep Eutectic Solvent) Ultrasound-assisted extraction (UAE)

The research design was created using Design Expert 7 software. A total of 48 conditions (triplo) were conducted, each of which constituted a comparative design with a

combination of HBA and HBD at a molar ratio of 1:5. These combinations included choline chloride and malic acid, as well as choline chloride and lactic acid. The NaDES solvents were prepared in accordance with the specified ratio, with the addition of 100% of the specified molarity to the mixture of solvent components. Additionally, the temperature ranged from 25 to 75 degrees Celsius, and the time span was between 3 and 30 minutes (23,37). Subsequently, the solution is separated via centrifugation for a period of 10 minutes at a speed of 5000 rpm. Following this, the sample is prepared for the calculation of luteolin, which has been successfully extracted. The extract solution is stored at room temperature (15,39,42,43).

Detection using UV-VIs spectrophotometers

The maximum wavelength of the luteolin comparator was identified (λmax = 350 nm). (44–46). DMSO was used as the solvent/blank for luteolin (47). Furthermore, a calibration curve was created using five variations of concentration with the standard compound luteolin. The identification of compounds successfully extracted for each treatment, designed based on NADES, was also completed. The initial identification process was carried out using a UV-Vis spectrophotometer.

Analysis of luteolin compound with HPLC-MS/MS instruments

Phytochemical analysis were carried out utilizing HPLC-MS/MS instruments utilizing. NaDES Extract result were arranged by the SPE (Solid Phase Extraction) strategy. The examination with HPLC-MS/MS was carried out with system column sort utilized was ACQUITY UPLC® HSS C18 (1.8 µm 2.1×100 mm, waters, USA) at temperatures of 50ºC (column) and 25ºC (room). HPLC-MS/MS examination was utilized eluent A that comprise of water and ammonium formate (99.9: 0.1), and eluent B that comprise of acetonitrile and formic acid (99.9:0.1) with a stream rate of 0.2 mL/min (gradient) for 23 min. (6,48–51). The results of HPLC-MS/MS were then analyzed using masslynx, msconvert and sirius software (6,52,61,53–60).

Data Analysis

Design Expert 7 software was used to design a comparison and determine the best % optimization for the extraction of luteolin compounds from celery (Apium graveolens) samples. The research variables were selected based on their impact on the process: Hydrogen Bonding Acceptor (choline chloride), Hydrogen Bonding Donor (lactic acid, malic acid), time, and temperature.

RESULTS

Calibration curve of luteolin compound

A linear regression equation Y = 0.0572x+0.1054 was obtained for the luteolin compound with an R value of 9973. The blank in this section was prepared using DMSO (47). This will be the definitive reference for calculating the levels of compounds obtained, along with the percentage of compounds obtained from the treatment that has been carried out. A wavelength of 350 nm was used for luteolin detection with a concentration series of 4 ppm, 6 ppm, 8 ppm, 10 ppm, and 12 ppm. (44–46)

Figure 1. Celery (apium graveolens)

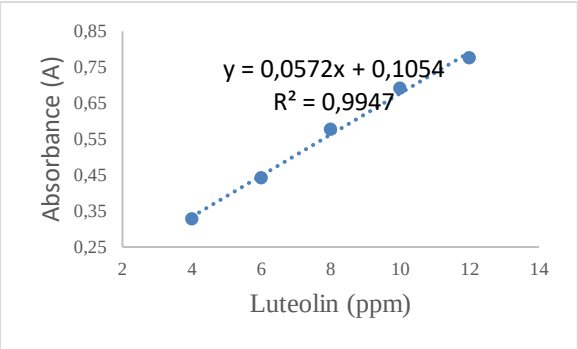

Figure 2. The calibration curve of the luteolin compound

NaDES Solvent

We then prepared the NaDES solvent and extracted the compounds using the ultrasonic method. We detected the filtrate with a UV-Vis spectrophotometer instrument and calculated the percentage of luteolin compounds that were successfully detected at a wavelength of 350 nm (44–46). DMSO was used as a blank for the detection of luteolin compounds. We used an acid-based NaDES solvent with a combination of choline chloride and lactic acid, as well as choline chloride and malic acid. The combination of choline chloride and lactic acid in the NaDES solvent produced promising results. The highest yield was achieved with a ratio of choline chloride to lactic acid that was almost exact. These findings were further validated by temperature and time effects. These results pave the way for the development of faster extraction methods.

We prepared the NaDES solvent by mixing the HBA, HBD, and distilled water components. We obtained a clear, transparent NaDES solvent within the first hour. Our observations ranged from 30 minutes to 45 minutes. We used a ratio of 0.1 in the number of moles of HBA and HBD components..

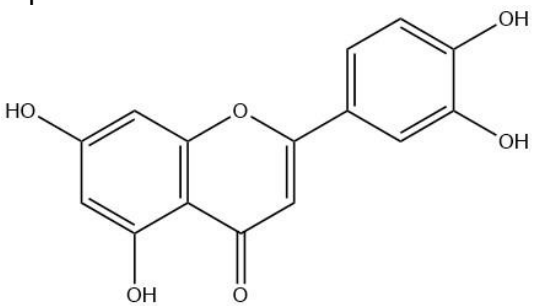

Figure 3. Luteolin

Table 1. Choline chloride : lactic acid

Run	HBA/ ChCl	HBD/ Lactic acid	Temperature	Time	% yield
1	5	5	25	30	0.3155
2	1	1	75	30	2.2492
3	1	1	75	30	2.2492
4	1	5	25	30	0.3640
5	5	5	25	3	0.1412
6	5	1	25	3	0.3640
7	1	5	25	3	0.3211
8	5	5	75	3	0.3145
9	1	1	25	3	1.4762
10	5	5	25	3	0.1412
11	1	1	75	30	2.2492
12	5	1	25	30	0.2118
13	5	5	75	30	0.2952
14	5	5	25	3	0.1412
15	1	5	75	30	0.5903
16	1	5	25	30	0.3648
17	5	1	75	30	0.5903
18	1	5	25	3	0.3203
19	1	1	75	3	1.8887
20	5	5	75	3	0.3145
21	5	1	25	30	0.5396
22	5	5	75	3	0.3155
23	5	1	25	3	0.2891
24	1	5	75	3	0.4288
25	5	1	75	3	0.2875
26	5	1	75	30	0.4288
27	5	1	25	30	0.4951
28	5	5	25	30	0.3160
29	5	5	25	30	0.3160
30	1	5	25	3	0.3203
31	5	1	75	3	0.4741
32	1	1	75	3	1.8887
33	1	5	75	30	0.5903
34	5	1	25	3	0.5396
35	5	5	75	30	0.2973
36	1	5	75	3	0.4296
37	1	1	25	30	1.4816
38	5	5	75	30	0.2968
39	1	1	75	3	1.8887
40	5	1	75	30	0.4452
41	1	1	25	3	1.4762
42	5	1	75	3	0.7207
43	1	5	75	3	0.4288
44	1	5	25	30	0.3664
45	1	1	25	30	1.4789
46	1	5	75	30	0.5903
47	1	1	25	3	1.4734
48	1	1	25	30	1.4789

Table 2. Choline chloride : malic acid

Run	HBA/ ChCl	HBD/ Lactic acid	Tempe rature	Time	% yield
1	5	5	75	30	0.0226
2	5	5	25	30	0.0108
3	1	1	25	3	0.0818
4	1	5	75	3	0.0277
5	5	5	75	3	0.0093
6	5	1	25	30	0.0158
7	5	1	25	3	0.0409
8	5	1	25	3	0.0409
9	5	5	75	3	0.0093
10	1	1	25	3	0.0821
11	1	1	25	30	0.0724
12	1	1	75	30	0.1317
13	5	5	25	3	0.0216
14	1	5	25	3	0.0423
15	1	5	75	30	0.0229
16	5	5	25	30	0.0108
17	1	5	25	30	0.0259
18	5	1	25	3	0.0408
19	1	5	25	3	0.0422
20	5	5	25	3	0.0216
21	5	1	75	30	0.0323
22	1	5	25	30	0.0259
23	5	5	75	3	0.0092
24	1	5	75	30	0.0229
25	1	1	75	3	0.0795
26	1	5	75	3	0.0278
27	1	1	75	3	0.0792
28	5	1	75	3	0.0157
29	1	1	25	3	0.0818
30	1	1	25	30	0.0727
31	1	1	75	30	0.1320
32	1	5	75	3	0.0277
33	5	1	75	30	0.0325
34	5	1	75	3	0.0159
35	5	5	75	30	0.0227
36	1	5	25	30	0.0256
37	5	5	25	30	0.0109
38	5	1	25	30	0.0159
39	1	1	75	3	0.0792
40	1	1	75	30	0.1320
41	1	5	25	3	0.0422
42	5	1	75	3	0.0159
43	5	5	25	3	0.0217
44	1	1	25	30	0.0727
45	1	5	75	30	0.0230
46	5	1	75	30	0.0325
47	5	5	75	30	0.0225
48	5	1	25	30	0.0158

Table 3. Optimation analysis by design expert 7 software

choline chloride	lactat acid	temp eratur e	time	Desir ability	% yield
4.71	4.76	55.24	15.63	0.278 8	2.28
choline chloride	malat acid	temp eratur e	time	Desir ability	% yield
1.27	1.71	35.45	12.73	0.257 6	0.01

The best optimization was obtained in the combination of NaDES solvent choline chloride: lactic acid in a ratio of 4.71 mol: 4.76 mol at a temperature of 55.24C and a time of 15.63 minutes. Under these conditions, 2.2789% is obtained with a desirability value of 0.278763107. The optimization gives a total volume of 217.2782 mL with 10% sample at a weight of 21.72782 gram. The best optimization was obtained in the combination of NaDES solvent choline chloride: malic acid in the ratio of 1.27 mol: 1.71 mol at a temperature of 35.45C and a time of 12.73 minutes. Under these conditions, 0.0102% is obtained with a desirability value of 0.040847856. The optimization gives a total volume of 81.32226 mL with 10% sample at a weight of 8.132226 gram.

The best optimization was obtained in the preparation of the best NaDES solvent in the combination of choline chloride: lactic acid, but the combination of choline chloride: malic acid was also successful for the extraction of the target compound luteolin.

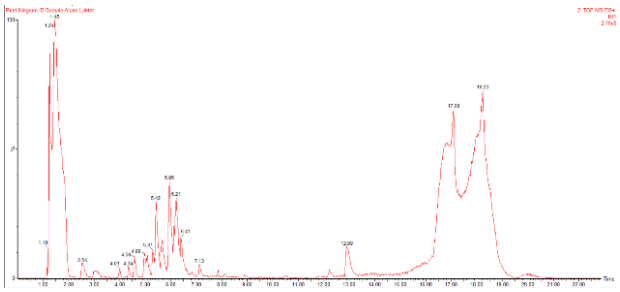

Figure 4. Masslyx software analysis for NaDeS extract for luteolin compound (of choline chloride: lactic acid)

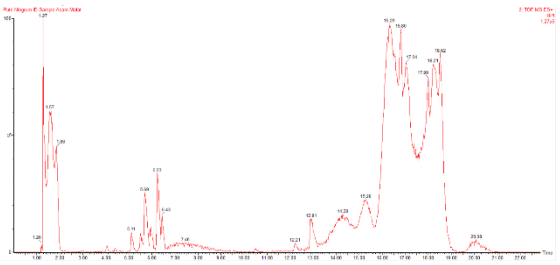

Figure 5. Masslyx software analysis for NaDeS extract for luteolin compound (choline chloride: malic acid)

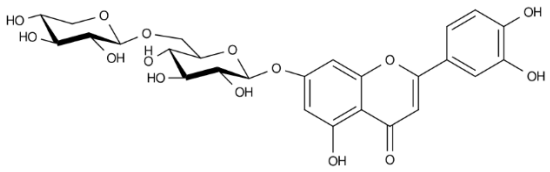

Figure 6. Luteolin 7-primeveroside

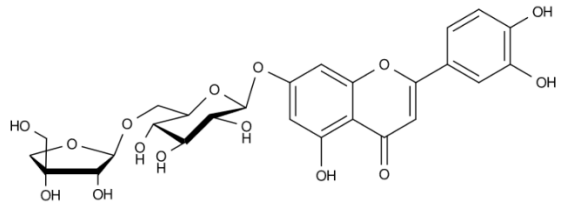

Figure 7. Luteolin 7-apiosyl(1->6)glucoside

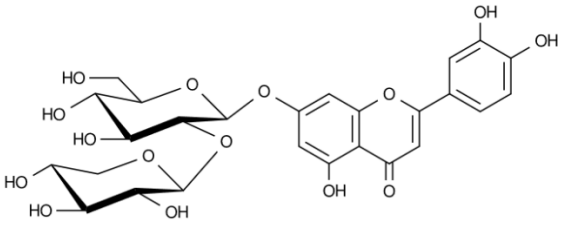

Figure 8. Luteolin 7-sambubioside

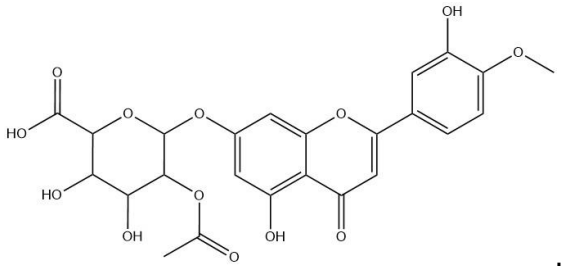

Figure 8. 5-acetoxy-3,4-dihydroxy-6-[5-hydroxy-2-(3-hydroxy-4-methoxy-phenyl)-4-oxo-chromen-7-yl]oxy-tetrahydropyran-2-carboxylic acid

In the results of the analysis of luteolin compounds using HPLC-MS/MS instruments, Luteolin derivative compounds were found. In the treatment with NaDES solvent choline chloride: lactic acid, the compounds Luteolin 7-primeveroside (Figure 6), Luteolin 7-apiosyl(1->6)glucoside (Figure 7) and Luteolin 7-sambubioside (Figure 8) were found. In the treatment with the solvent NaDES choline chloride: malic acid, the compound 5-acetoxy-3,4-dihydroxy-6-[5-hydroxy-2-(3-hydroxy-4-methoxy-phenyl)-4-oxo-chromen-7-yl]oxy-tetrahydropyran-2-carboxylic acid was found. In this compound the basic structure of the luteolin compound was found, but with side chains that have not been further identified. It is possible that this is a new compound that has not been widely discussed in various fields of research.

CONCLUSIONS

The NaDES solvent synthesis of choline chloride: lactic acid by ultrasonic treatment has successfully obtained % luteolin compounds at more than 2%. The combination of choline chloride: malic acid was still successfully extracted. Detection of compounds using UV-Vis spectrophotometer instrument at a wavelength of 350 nm.

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