CHEMICAL ENGINEERING TRANSACTIONS
VOL. 106, 2023

A publication of
AIDIC
The Italian Association
of Chemical Engineering
Online at www.cetjournal.it

Guest Editors: Jeng Shiun Lim, Nor Alafiza Yunus, Peck Loo Kiew, Hon Huin Chin

ISBN 979-12-81206-05-2; **ISSN** 2283-9216

DOI: 10.3303/CET23106109

Ultrasonic-assisted Extraction to Recover Phenolics from *Paris Polyphylla*

Tan Phat Vo[a,b], Thi Hoang Yen Nguyen[a,b], Khanh Vi Truong[a,b], Ngoc Van Anh Hoang[a,b], Ngoc Minh Khang Le[b], Dinh Quan Nguyen[a,b,*]

[a]Laboratory of Biofuel and Biomass Research, Faculty of Chemical Engineering, Ho Chi Minh City University of Technology (HCMUT), 268 Ly Thuong Kiet Street, District 10, Ho Chi Minh City, Vietnam
[b]Vietnam National University Ho Chi Minh City, Linh Trung Ward, Thu Duc City, Ho Chi Minh City, Vietnam
ndquan@hcmut.edu.vn

In this research, optimize ultrasonic-assisted extraction is aimed to obtain phenolics from Paris polyphylla using solvents: Glucose-Lactic acid, Choline-Lactic acid, Glycerol-Lactic acid, Sodium-Lactic acid, Choline-Citric acid, Glycerol-Citric, Sodium-Citric acid, Glucose-Citric acid, and the solvent with the highest extraction capacity for phenolics is Glucose-Lactic acid. Then, single-factor experiments were carried out to study the effect of the process parameters of ultrasonic-assisted extraction on the extraction efficiency of phenolics. The center point response surface model is used to optimize the extraction process with ultrasonic-assisted waves for phenolics recovery from P. polyphylla. The optimal conditions of terpenoids were 450 W of microwave power, 20 % of water content, and 50 mL/g of liquid-to-solid ratio. The results obtained from the model are close to the experimental results, proving that the optimized model is suitable for predicting experimental results. The study provided a green and sustainable approach to acquire bioactive compounds from P. polyphylla .

1. Introduction

Paris polyphylla, often referred to as "chirayita" or "medicinal Paris," is a perennial herb that is found in China, India, Nepal, and Myanmar and is a member of the Trilliaceae family of plants. The tree can be found in Vietnam in Moc Chau (Son La), Dong Van (Ha Giang), and other locations (Geng et al., 2018). It has long been used in traditional medicine to treat a variety of illnesses, including fever, inflammation, pain, and diabetes (Geng et al., 2018). Many substances found in the plant, including saponins, flavonoids, and phenolic acids, are thought to have anti-inflammatory and anti-diabetic characteristics (Geng et al., 2018). Free radicals are unstable chemicals that can damage cells and contribute to the onset of several chronic diseases, including cancer, cardiovascular disease, and diabetes. Antioxidants are molecules that counteract these negative effects (Ferreira et al., 2007). Anti-diabetic agents, on the other hand, help regulate blood sugar levels and reduce the risk of complications associated with diabetes, such as neuropathy, retinopathy, and nephropathy (Lepcha et al., 2019). Several studies have investigated the antioxidant and anti-diabetic properties of *P. polyphylla* extracts (Huang et al., 2022).

Ultrasound-assisted extraction (UAE) u uses high-frequency sound waves to remove chemicals from a sample. Because of cavitation effects, the solvent penetrates the sample and releases the desired components. UAE has been demonstrated to be a quick, effective, and eco-friendly extraction technique that works with a range of sample types and solvents (Ramsay et al., 2017). The foundation of UAE is the application of high-frequency sound waves to the sample matrix to cause disruption and encourage the release of the target chemicals. This method uses acoustic cavitation when small bubbles develop and implosive collapse in a liquid medium (Nawaz et al., 2020). When these bubbles burst, shock waves and micro-jets are produced, which cause shear forces and stresses in the sample and cause the target chemicals to release (Tranchida et al., 2018).

NADES (Natural Deep Eutectic Solvents) are a kind of solvent that consists of natural, renewable and biodegradable components, which makes them more environmentally friendly than conventional synthetic solvents that can be damaging to the environment. NADES solvents can also be created in a laboratory setting

Paper Received: 30 May 2023; Revised: 08 August 2023; Accepted: 29 August 2023
Please cite this article as: Vo T.P., Nguyen T.H.Y., Truong K.V., Hoang N.V.A., Le N.M.K., Nguyen D.Q., 2023, Ultrasonic-assisted Extraction to Recover Phenolics from Paris polyphylla, Chemical Engineering Transactions, 106, 649-654 DOI:10.3303/CET23106109

by blending natural compounds together to produce an eutectic mixture that has a lower melting point than individual components. Natural compounds like sugar, amino acid, and choline chloride can all be utilised to produce NADES solvents (Abbott et al., 2004). NADES-based UAE is a method of extracting bioactive compounds from plant material using NADES and ultrasound waves. It has been used to extract a wide range of bioactive compounds from various plants including essential oils, phenolic compounds, flavonoids and alkaloids. For example, a study published in the Journal of Food Science and Technology in 2019 reported the extraction of phenolic compounds from grape pomace using UAE (Romanini et al., 2021). Researcher using a NADES solvent composed of choline chloride and lattice acid to extract the phenolic compounds (Romanini et al., 2021). However, there are currently no studies on phenolic extraction from *P. polyphylla* using UAE(Romanini et al., 2021).

This study aims to maximise the NADES-based UAE extraction conditions to obtain the greatest extraction yields of phenolics simultaneously. This study aimed to optimise the ultrasonic-assisted extraction process combined with NADES solvent to obtain phenolics from *P.polyphylla* . This study can shed important light on the conditions of NADES-based UAE for extracting bioactive compounds from *P. polyphylla* using response surface methodology. Univariate experiments were carried out to investigate the effect of ultrasound on the phenolics recovery efficiency and the center surface response model was selected to optimise the ultrasonic assisted extraction for optimal phenolics recovery. This is a new study that is applied and optimized in the process of extract phenolics through UAE and NADES to reduce time, minimize solvent usage, and improving yield (Mathialagan et al., 2016).

2. Material and methods

2.1 Clarity

Paris polyphylla were purchased from Thanh Binh Herbal Tea Company, Ho Chi Minh, Vietnam, and their roots of them were sliced into small pieces. These pieces were dried at 45 °C for 45 h to obtain a moisture content of 8 % grounded to obtain Paris polyphylla root powder (PRP). Absolute ethanol (purity ≥ 99.8 %), acetone (purity ≥ 99.5 %), potassium acetate (purity ≥ 99.5 %), sodium carbonate (purity ≥ 99.5 %), aluminum chloride hexahydrate (purity 99 %), hydrochloric acid (purity ≥ 36.5 %), potassium acetate (purity ≥ 99 %), potassium chloride (purity ≥ 99 %), sodium acetate (purity ≥ 99.5 %), Folin–Ciocalteu reagent (concentration 1.9–2.1 N), gallic acid monohydrate (purity ≥ 98 %) were acquired from Sigma-Aldrich Chemical Co., Ltd, Singapore, Singapore. The chemicals used for NADES preparation were obtained from Xilong Scientific Co Ltd, Guangdong, China.

2.2 NADES preparation and the screening of solvents

In this study, six natural deep eutectic solvents were prepared using the heating method. The HBAs and HBDs were blended in an appropriate molar ratio, heated at 90 °C, and stirred using a magnetic stirrer (model: C–MAG HS 7, IKA Industrie, Humboldtstraβe, Konigswinter, Germany). This process was completed when a clear and homogeneous liquid formed. The chemicals, abbreviations, and molar ratios for NADES preparation are expressed in Table 1.

PRP weighed 0.5 g and was dispersed into 10 mL of the prepared NADES (20 % water content, g/g) in 100 mL amber glass bottles. The mixture was sonicated using an Elmasonic ultrasonic bath (S300H, Elma Schmidbauer, Gottlieb-Daimler-Straße, Hohentwiel, Germany) with 300 W, ultrasonic power at room temperature (30 °C) for 5 min. After sonication, the mixtures were centrifuged (DM0412, DLAB Science Co., Ltd, Shunyi, Beijing, China) at 4,000 rpm for 20 min, distilled water was used as control solvents. The Phenolic composition of the extract has been quantified.

Table 1: NADES used in this research

No.	Abbreviation	HBD	HBA	Molar Ratio
1	Glu-Lac	D-Glucose	Lactic acid	2:1
2	Choline - Lac		Lactic acid	2:1
3	Gly-Lac	Glycerin	Lactic acid	2:1
4	Sodium-Lac	Sodium acetate	Lactic acid	2:1
5	Cho-Ci		Citric acid	2:1
6	Gly-Ci	Glycerin	Citric acid	2:1
7	So-Ci	Sodium acetate	Citric acid	2:1
8	Glu-Ci	D-Glucose	Citric acid	2:1

2.3 Single-factor experiments

PRP was sonicated at different LSRs (10–100 mL/g) with varying contents of the water in NADES (10, 20, 30, 40, 50 % g/g) at temperatures 30 °C for different ultrasonic powers (0,150, 300, 450, 600, 750, 900 W). After sonication, the mixtures were centrifuged at 4,000 rpm for 20 min to obtain extracts. The extracts' total phenolic content (TPC) was measured using the methods in Section 2.5.

2.4 Design of experiments

A BBD model, which was used to find optimal conditions and parameter interaction, was conducted based on the experimental values of one-factor experiments. Regarding UAE, three factors (LSR, ultrasonic power, and water content) with three levels (-1, 0, +1) were employed to explore the linear and interactive effects on the extraction yield of phenolics. The dependent responses for optimizing the UAE process were TPC. Analysis of variance (ANOVA) analyzed the significance of process parameters.To verify the correctness of the model, the experiment is performed under optimal conditions. Optimal conditions and theoretical phenolic content were found from the model to compare experimental results.

2.5 Determination of the total phenolic content

The TPC was measured using the Folin-Ciocalteu reagent. TPC was presented as gallic acid equivalent milligrams per gram of dried basis weight (mg GAE/g).

2.6 Statistical analysis

All experiments were repeated three times and expressed as the mean ± SD. Analysis of variance (ANOVA) with 95 % confidence and other statistical analysis was analyzed by Minitab 19 (Minitab, Inc, Pennsylvania, USA). The BBD model was analyzed utilizing Design-Expert v.13 software (Stat-Ease Inc., Minneapolis, Minnesota, USA). Graphics were constructed by Origin Pro (Origin Lab, Northampton, Massachusetts, USA).

3. Result and discussions

3.1 Assessing the extraction efficiency of NADES

The efficiency of using NADES-based UAE was evaluated using TPC at a ratio of 20 mL/g LSR, a temperature of 30°C, and water content of 20 % for a retention time of 5 min. NADES was created by combining hydrogen bond donors, primarily lactic acid, with hydrogen bond acceptors such as glucose. The recovered TPC was compared to results obtained using traditional solvents, and the findings are shown in Figure 1a. Among all NADES tested, the extraction performance of Glu-Lac was the highest and obtained TPC reached the peak at 3.34 mg GAE/g dw, which was the highest. The superior performance of Glu-Lac can be attributed to its similar polarity to the phenolics found in PRP (Rashid et al., 2023). Glu-Lac was selected for further experimentation in this study.

3.2 Effect of the liquid-to-solid ratio

Figure 1b presents the variation of TPC in the NADES-based UAE process when LSR increased from 10 to 100 mL/g. TPC increased by 1.57 times as the LSR rose from 10 to 50 mL/ g. Increasing LSR can improve the contact area between Glu- Lac and PRP (Zheng et al., 2022). The increase in ultrasonic intensity enhances the cavitation effect by lowering the cavitation threshold (Patil, et al. 2021). As the LSR increases, the viscosity decreases, this leads to reducing the energy required to create cavitation bubbles and increases the number of cavitation bubbles (Patil et al., 2021). When the cavitation bubbles burst, shear forces are generated on the surface of the material, creating micro-channels that increase the efficiency of the process (Rao et al., 2021). The appropriate LSR was 50 mL/g to attain the highest extraction efficiency of phenolics from PRP.

3.3 Effect of the water content in NADES

The influence of the water content in Glu- Lac from 10 % to 50 % on TPC, was analyzed under ultrasonic impact, and the results are presented in Figure 1c. As shown in Figure 1c, TPC escalated by 1.6 times when the water content increased from 10 % to 20 %. Adding water to Glu- Lac can increase surface tension by raising the number of hydrogen bonds. The increased hydrogen bonds can strengthen an attractive force against an external one, leading to a strong mutual attraction on the NADES surface (*Synthesis of Citric Acid Monohydrate-Choline Chloride Based Deep Eutectic Solvents (DES) and Characterization of Their Physicochemical Properties - ScienceDirect*, n.d.). This phenomenon can improve the solubility of phenolics in Glu- Lac, promoting extraction efficiency. Excessive water addition can disintegrate the hydrogen-bonding networks and make significant differences in polarity among Glu-Lac and phenolics, thus decreasing extraction efficiency (Huang, et al. 2022). This result agrees with Zeng et al. (2019), that used NADES-grounded UAE to prize phenolics from Chinese wild rice (Zeng et al., 2019). The appropriate water content in Glu- Lac was 20 % to obtain the highest extraction efficiency of phenolics from PRP.

3.4 Effect of power

Power contributes to extraction efficiency due to the varying ultrasonic intensity of solvents (Zheng et al., 2022). The effect of power on the NADES-based UAE of phenolics and flavonoids was investigated in the range of 0 - 900 W, and the results are demonstrated in Figure 1d. As shown in Figure 1d, the recovery of TPC displayed a significant increase of 2.37 times when the power increased 0 to 450 W. An increase in power can increase the number and size of cavitation bubbles, resulting in increased cell wall destruction, increasing the diffusion of the solvent into the tissue matrix. An increased diffusion can improve the mass transfer rate and enhance the desorption properties, improving phenolics extraction efficiency in PRP (Kumar et al., 2021).TPC decreased by 1.31 times, when power increased from 450 - 900 W. Additionally, the high power can inverse influence the cavitation effects, which can cause less damage to PRP cell walls. The ultrasonic power chosen to use in this study was 450 W.

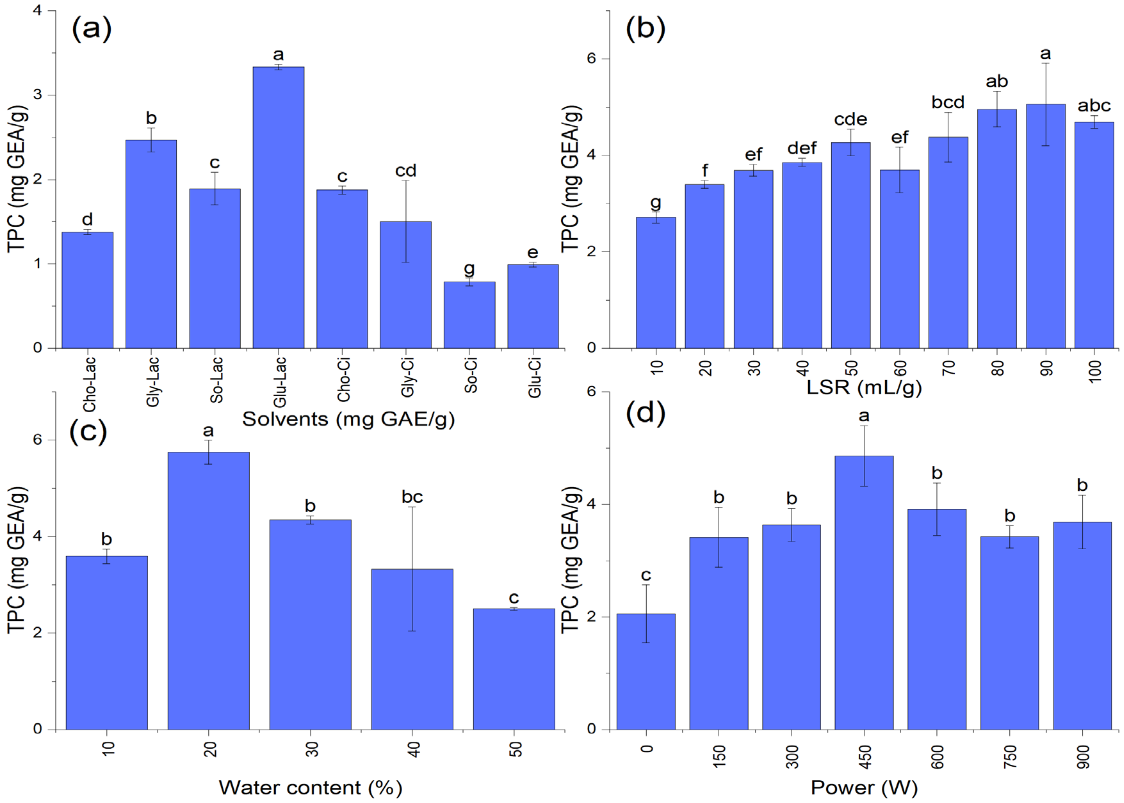

Figure 1: Influences of samples on the extraction efficiency of phenolics PRP extracts at fixed conditions: 30 °C, and retention time 5 min.: (a): Solvents (fixed conditions: 20 mL/g, ultrasonic power 300 W, 20 % water content, 30 °C and 5 min), (b): SLR (fixed conditions: ultrasonic power 300 W, 20 % water content, 30 °C and 5 min), (c): Water content (fixed conditions: 80 mL/g, ultrasonic power 300 W, 30 °C and 5 min), (d) Power. (fixed conditions: 80 mL/g, 20 % water content, 30 °C and 5 min). Different characters: a, b, and c presented significant statistical differences.

4. Regression models

The variables and dependent responses of RSM with BBD models are presented in Table 2, and the regression coefficients are shown in Table 2. After analyzing the multiple regression of experimental results, a second-order polynomial model was employed to present the suggested model. The second-order polynomial models Eq (1) expressed a correlation between the variables and the dependent responses shown in Table 2.

$$Y = 4.86 + 0.3079X_1 - 0.189X_2X_1 - 0.3677X_2X_3 - 0.3359X_1^2 - 0.3256X_2^2 - 0.2201X_3^2 \qquad (1)$$

Table 2: The result of experimental design and analysis of variance of regression coefficients

Run	LSR	Water Content	Ultrasonic Power	TPC mg GAE/g		Regression Coefficients	p-value	F-value
1	0	-1	1	4.287	Intercept	4.86	0.0006	63.83
2	0	1	-1	5.083	A-A	0.05	0.0814	5.37
3	1	0	-1	4.49	B-B	0.3079	0.0001	203.65
4	0	-1	-1	3.726	C-C	-0.0541	0.0661	6.29
5	-1	-1	0	3.685	AB	-0.189	0.0035	38.37
6	0	0	0	4.863	AC	-0.0845	0.0504	7.67
7	0	0	0	4.863	BC	-0.3677	0.0003	145.28
8	1	-1	0	4.108	A^2	-0.3359	0.0006	96.95
9	-1	0	-1	4.166	B^2	-0.3256	0.0007	91.12
10	1	1	0	4.34	C^2	-0.2201	0.003	41.64
11	0	1	1	4.173	R^2	0.9931		
12	1	0	1	4.279	Adjusted R^2	0.9775		
13	-1	1	0	4.673	Predicted R^2	0.8894		
14	-1	0	1	4.293	Adeq Precision	26.9986		

The ANOVA and regression models were constructed using Design Expert 13. The results of ANOVA can demonstrate the confidence of the second-order polynomial models. As illustrated in Table 2, it was clear that the models were highly significant ($p < 0.01$) for the measurement of TPC. Additionally, the coefficient of determination (R_2 = 0.9931) was higher than 0.9, and the coefficient of adjusted determination (Adjusted R_2 = 0.9775) for TPC respectively were close to the values of determination coefficients, implying that the models were reliable to show the correlation between variables and dependent responses. All the results verified that the second-order polynomial models could match the experimental results and were appropriate for the anticipation of independent responses. 3D response surface graphics (Figure 2) was built to demonstrate the interactive effect of responses and factors. LSR and water content had interactive effects on TPC ($p < 0.05$). In terms of interactive effect, LSR, water content, and ultrasonic power had a negative effect on TPC. The experiment was conducted at optimal conditions (50 mL/g LSR, 30 % water content, and 300 W) to validate the regression model. The experimental data of TPC at optimized UAE conditions was 5.67±0.47 mg GAE/g, which was close to the predicted values of the model. It can be concluded that the regression model can be suitable for predicting experimental results

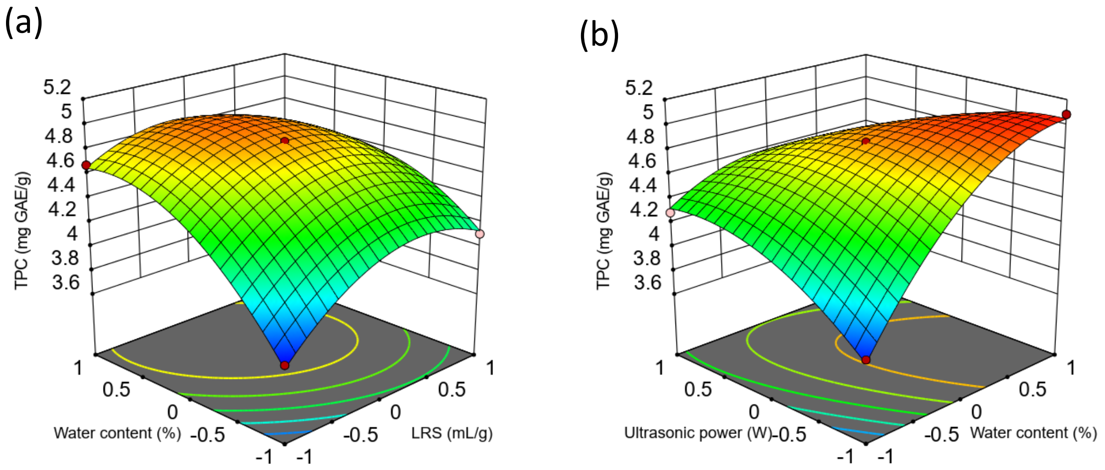

Figure 2: The significant interactive effect of response on TPC ; (a): interactive effect of water content and LSR on TPC; (b): interactive effect of ultrasonic power and water content on TPC

5. Conclusions

In this study, Glu- Lac was a more effective solvent than water (0.73 ± 0.06 mg GAE/g), solutions for extracting phenolics from PRP. The best Glu-Lac-based UAE conditions were 50 mL/g LSR, 20 % water content and 450 W to read the yield 5.05 mg GAE/g of TPC. The study highlights that Glu-Lac-based UAE offers several advantages, making it an environmentally friendly and efficient method for extracting bioactive compounds from PRP. This finding opens up new possibilities for utilizing Glu-Lac as a green and effective alternative to traditional extraction methods in the pursuit of bioactive compounds from natural sources.

Acknowledgments

We acknowledge Ho Chi Minh City University of Technology (HCMUT), VNU-HCM for supporting this study.

References

Abbott A.P., Boothby D., Capper G., Davies D.L., Rasheed R.K., 2004, Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids, Journal of the American Chemical Society, 126(29), 9142-9147.

Ferreira I., Rocha S., Coelho M., 2007, Encapsulation of Antioxidants by Spray-Drying, Chemical Engineering Transactions, 11(2), 713-717.

Geng C.A., Yang T.H., Huang X.Y., Yang J., Ma Y.B., Li T.Z., Zhang X.M., Chen J.J., 2018, Anti-hepatitis B virus effects of the traditional Chinese herb Artemisia capillaris and its active enynes, J Ethnopharmacol, 224, 283-289.

Huang H., Zhu Y., Fu X., Zou Y., Li Q., Luo Z., 2022, Integrated natural deep eutectic solvent and pulse-ultrasonication for efficient extraction of crocins from gardenia fruits (Gardenia jasminoides Ellis) and its bioactivities, Food Chemistry, 380, 132216.

Kumar K., Srivastav S., Sharanagat V.S., 2021, Ultrasound assisted extraction (UAE) of bioactive compounds from fruit and vegetable processing by-products: A review, Ultrason Sonochem, 70, 105325.

Lepcha D.L., Chhetri A., Chhetri D.R., 2019, Antioxidant and Cytotoxic Attributes of Paris polyphylla Smith from Sikkim Himalaya, Pharmacognosy Journal, 11(4), 705-711.

Mathialagan R., Mansor N., Shamsuddin M.R., Uemura Y., Majeed Z., 2016, Extraction of Allicin from Garlic Using Ultrasonic-Assisted Method, Chemical Engineering Transactions, 56, 1747-1752.

Nawaz H., Shad M.A., Rehman N., Andaleeb H., Ullah N., 2020, Effect of solvent polarity on extraction yield and antioxidant properties of phytochemicals from bean (Phaseolus vulgaris) seeds, Brazilian Journal of Pharmaceutical Sciences, 56.

Patil S.S., Pathak A., Rathod V.K., 2021, Optimization and kinetic study of ultrasound assisted deep eutectic solvent based extraction: A greener route for extraction of curcuminoids from Curcuma longa, Ultrasonics Sonochemistry, 70, 105267.

Ramsay A., Fliniaux O., Quéro A., Molinié R., Demailly H, Hano C., Paetz C., Roscher A., Grand E., Kovensky J, Schneider B., Mesnard F., 2017, Kinetics of the incorporation of the main phenolic compounds into the lignan macromolecule during flaxseed development, Food Chemistry, 217, 1-8.

Rao M.V., Sengar A.S., Sunil C.K., Rawson A., 2021, Ultrasonication - A green technology extraction technique for spices: A review, Trends in Food Science & Technology, 116, 975-991.

Rashid R., Wani S.M., Manzoor S., Masoodi F.A., Dar M.M., 2023, Green extraction of bioactive compounds from apple pomace by ultrasound assisted natural deep eutectic solvent extraction: Optimisation, comparison and bioactivity, Food Chem, 398, 133871.

Romanini E.B., Rodrigues L.M., Finger A., Chierrito T.P.C., Scapim M.R.S., Madrona G.S., 2021, Ultrasound assisted extraction of bioactive compounds from BRS Violet grape pomace followed by alginate-Ca2+ encapsulation, Food Chemistry, 338, 128101.

Tranchida P.Q., Aloisi I., Giocastro B., Mondello L., 2018, Current state of comprehensive two-dimensional gas chromatography-mass spectrometry with focus on processes of ionization, TrAC Trends in Analytical Chemistry, 105, 360-366.

Zeng J., Dou Y., Yan N., Li N., Zhang H., Tan J.N., 2019, Optimizing Ultrasound-Assisted Deep Eutectic Solvent Extraction of Bioactive Compounds from Chinese Wild Rice, in Molecules, 24(15), 2718.

Zheng B., Yuan Y., Xiang J., Jin W, Johnson J.B., Li Z., Wang C., Luo D., 2022, Green extraction of phenolic compounds from foxtail millet bran by ultrasonic-assisted deep eutectic solvent extraction: Optimization, comparison and bioactivities, LWT, 154, 112740.