International Journal of Applied Pharmaceutics

ISSN- 0975-7058 Vol 16, Special Issue 5, 2024

Original Article

OPTIMIZATION ULTRASOUND-ASSISTED EXTRACTION USING CHOLINE CHLORIDE-BASED NATURAL DEEP EUTECTIC SOLVENT TO INCREASE PHENOLIC COMPOUNDS AND ANTIOXIDANTS FROM RHODOMYRTUS TOMENTOSA LEAVES

NINA D. OKTAVIYANTI[1], RYANTO BUDIONO[2], ENDANG W. FITRIANI[3], CHRISTINA AVANTI[3*]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Surabaya, Surabaya-60293, East Java, Indonesia. [2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Surabaya, Surabaya-60293, East Java, Indonesia. [3]Department of Pharmaceutical, Faculty of Pharmacy, Universitas Surabaya, Surabaya-60293, East Java, Indonesia
[*]Corresponding author: Christina Avanti; [*]Email: c_avanti@staff.ubaya.ac.id

Received: 06 May 2024, Revised and Accepted: 25 Aug 2024

ABSTRACT

Objective: The main purpose of this study is to give recommendations for the ideal extraction conditions for improving the extraction yield and antioxidant activity of *R. tomentosa* leaves.

Methods: First, the extraction total phenolic yields of five choline chloride-based Natural Deep Eutectic Solvents (NADES) were evaluated. Then, Box Behnken designs of Response Surface Methodology (RSM) were conducted in order to optimize the extraction condition. The extraction variables investigated were extraction time, water content in NADES, and solid-to-liquid ratio. Meanwhile, total phenolic and 2,2′-azinbis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radicals scavenging activity were used as responses.

Results: NADES with combination of choline chloride and propylene glycol with a molar ratio of 1:1 was found to be the best solvent for extracting phenolic compound. The total phenolic compound obtained was 29.6351 mg GAE/g dried leaves with ABTS scavenging activity about 96.84% under the optimum extraction condition (extraction time of 60 min, 25% water content in NADES, and solid-to-liquid ratio of 0.02 g/ml). Better results were shown compared to extracts with conventional solvents.

Conclusion: In sum, the use of choline chloride-based NADES as extraction solvent under optimum condition was proven to be effective in increasing the extraction efficiency of phenolic compounds and antioxidant activity from *R. tomentosa* leaves.

Keywords: *Rhodomyrtus tomentosa* leaves, Natural deep eutectic solvent, Response surface Methodology, Phenolic, Antioxidant

DOI: https://dx.doi.org/10.22159/ijap.2024.v16s5.52479 Journal homepage: https://innovareacademics.in/journals/index.php/ijap

INTRODUCTION

Rhodomyrtus tomentosa is flowering small tree plant a member of the family Myrtaceae. In Indonesia, especially Kalimantan, it is known as Karamunting. Its fruit is widely used as traditional medicine, and its compounds and biological activities have been studied [1]. The continuous availability of raw materials presents unique challenges for the preparation of herbal medicines using fruit parts as raw materials. Therefore, the leaf part was selected to be developed in this study as a promising raw materials candidate for herbal medicine. The potential of plant secondary metabolite compounds as antioxidants was demonstrated by our earlier research [2]. Phenolic compounds are the largest group of compounds found in plants and have been proven to be responsible for the biological activity of plants as herbal medicines and cosmetics [3]. There are many factors that influence the phenolic compounds obtained from a plant, including the extraction method used, type of solvent, extraction time and temperature, and many other extraction variables [4]. The different phenolic compound yields in a plant extract due to different extraction conditions affect the antioxidant capacity [5].

Thus far, organic solvents have been used for *R. tomentosa* leaves extraction in several studies [6, 7]. The use of volatile organic solvents in the extraction process can have negative impacts on the environment [8-11]. Our previous study succeeded in optimizing environmentally friendly extraction methods using Natural Deep Eutectic Solvent (NADES), which can increase extraction yields two times higher than ethanolic extract [2, 12, 13]. The use of green solvents as an alternative to organic solvents is included in the application of green extraction principles. NADES, a type of green solvent, have recently attracted a lot of attention because of their several advantages, including their effectiveness as an alternative solvent to replace organic solvents in the extraction process and as a raw material for cosmetics [14, 15]. NADES are formed from a mixture of hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) in certain molar ratios. Numerous studies have demonstrated the high effectiveness of choline chloride in plant metabolite extraction, which has led to its widespread use as HBD for NADES. It may be possible to formulate the NADES-containing extract straight away without first undergoing a solvent separation procedure, so it can be applied as a strategy for reducing the amount of energy and excipients utilized.

It is important to optimize extraction conditions using the suitable solvent for each plant in order to get the highest extraction yield [4]. In the current study, the Response Surface Methodology (RSM) has been used for designing, analyzing, and forecasting the extraction conditions. One benefit of RSM is that it can be used to predict the ideal extraction condition and comprehend how different extraction factors relate to one another.

Ultrasound-assisted extraction (UAE) is a non-conventional extraction method that is often used in green extraction applications. The cavitation phenomenon caused by ultrasonic waves facilitates increased contact between solvent and sample, increases the efficiency of extraction time solvent use, and reduces energy consumption so that extraction results can be improved [16]. This can support the application of the green extraction principle, which uses environmentally friendly extraction methods with minimal energy use.

To the best of our knowledge, study optimizing the extraction of *R. tomentosa* is still limited, and only its fruit. Moreover, the publications that optimize the extraction of Karamunting leaves using NADES solvent have never been reported elsewhere. The aim of this research was to investigate the ability of NADES-UAE to increase the extraction efficiency of *R. tomentosa* leaves and recommend the optimal extraction conditions that provide the highest total phenolic and antioxidant activity simultaneously.

MATERIALS AND METHODS

Materials

All of the HBA and HBD compound used for the extraction in this study were pharmaceutical grade, included choline chloride (Xi'an Rongsheng Biotechnology Co, Ltd, China); propylene glycol, sorbitol, glycerol, polyethylene glycol 400 (Merck, Germany). Meanwhile, 2,2′-azinbis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and Gallic acid were purchased from Sigma Aldrich, USA.

Preparation of NADES

In this research, NADES were prepared using a heating method where choline chloride as HBA was stirred with various types of HBD with appropriate molar ratios. This was carried out constantly at a temperature of 50 °C for 30 min until a stable, clear and homogeneous solution was formed. All the choline chloride-based NADES used in this study and their abbreviations and molar ratios are listed in table 1.

Table 1: List of NADES used in this study with the abbreviation

Combination of HBA and HBD	Molar ratio	Abbreviation
Choline chloride-Propylene glycol	1:1	ChPg1
Choline chloride-Propylene glycol	1:4	ChPg4
Choline chloride-Sorbitol	1:2	ChSb
Choline chloride-Glycerol	1:2	ChGl
Choline chloride-polyethylene glycol 400 (PEG400)	1:2	ChPeg

Sample collection and extract preparation

The *R. tomentosa* leaves used in this study were collected from Palangkaraya, Central Kalimantan, Indonesia. The authenticity of the sample was verified by the Center for Traditional Medicine Information and Development, Faculty of Pharmacy, University of Surabaya. The fresh leaves were sorted, washed, and dried in the shade, then sifted using a 30 mesh to obtain dried leaf powder. The powdered leaves preserved in a dry environment and sealed container for later use.

The UAE method was conducted to obtain all the *R. tomentosa* extract in our current study. Initially, the selection of NADES was carried out to choose the best solvent for extraction optimization. In this stage, about 0.5 g of dried leaves were sonicated with 10 ml NADES for 20 min at room temperature with a frequency of 40 kHz. Meanwhile, at the extraction optimization stage, *R. tomentosa* leaves were sonicated with a solid-to-liquid ratio, extraction time, and water content, which have been determined using RSM analysis. All the extract obtained were centrifuged at 1500 rpm for 15 min, and the filtrates were collected. Every extraction process used in this study was carried out three times.

Experimental design for optimization using the response surface method (RSM)

A Box-Behnken design (BBD) of the response surface method using three factors with three levels of each factor has been carried out to optimize the phenolic compounds and antioxidant activity of *R. tomentosa* leaves. The selected levels of three factors and their codes in this study are represented in table 2. A total of 15-run experiments ordered by RSM to be carried out for experimental verification. The final result of the Box-Behnken design is to formulate a second-order polynomial regression model, which is then used to predict optimal extraction conditions for phenolic compounds and antioxidant activity from *R. tomentosa* leaves.

$$Y = \beta_0 + \sum_{j=1}^{3} \beta_j X_j + \sum_{j=1}^{3} \beta_{jj} X_j^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

Where Y is the response variable (total phenolic content or antioxidant activity); β_0 is a constant and represents the intercept; βj, βj, βij are the linear, squared and interaction coefficients, respectively [17].

Table 2: The code, range, and level of each variable

Variables	Code	Range and level (xi)		
		-1	0	1
Extraction Time (min)	X_1	20	40	60
Water Content in NADES (%)	X_2	20	25	30
Solid-to-liquid ratio (g/ml)	X_3	0.02	0.05	0.10

Determination of total phenolic content (TPC)

The total phenolic content of the *R. tomentosa* extracts was determined spectrophotometrically (UV-1900, Shimadzu Corp., Kyoto) using the Total Phenolic Index (TPI) method, which refers to the method of Aleixandre-Tudo *et al.* [18] with slight modifications. About 0.5 ml of each extract filtrate was pipetted quantitatively into a 10.0 volumetric flask, and then water was added until the total volume. After being homogenized, all the solutions were read at a wavelength of 280 nm as the total phenolic index (TPI) equivalent to gallic acid. The total phenolic content was calculated using a standard curve of Gallic acid with a concentration ranging from 10 to 50 mg/l with y = 0.0245x – 0.0609 (R^2 = 0.9996). Total phenolic level was analyzed in triplicate and expressed as mg GAE per g dried leaves (mg GAE/g dried leaves).

Evaluation antioxidant activity

Antioxidant activity becomes a measurable parameter in extraction optimization procedures. The antioxidant activity was evaluated using the ABTS method according to previous studies conducted by Jacob *et al.* [19] and Oktaviyanti *et al.* [13] with modifications. Firstly, ABTS free radical was prepared by mixing and dissolving 7.1 mg ABTS and 3.5 mg $K_2S_2O_8$ in deionized water until a total volume of 25.0 ml. This mixture turned into an ABTS free radical solution after 16 h of incubation. Each extract solution was pipetted about 160 µl**, added with 40 µl** of ABTS free radical solution, and then incubated for 5 min at room temperature and then measured at 730 nm using microplate reader (BMG Labtech, Germany). All the antioxidant activity were calculated as percentage inhibition using equation below and performed in triplicate.

$$\% \text{ inhibition} = \frac{A - B}{A} \times 100\%$$

Where A is free radicals solution absorbance; B is the sample absorbance after mixing and incubating with free radicals solution.

Statistical analysis

Total phenolic level data obtained at the initial stage of NADES screening were compared by analysis of variance (ANOVA) with a significance level of $p<0.05$ using SPSS software version 18 for Windows (IBM, New York, United States).

Design Expert Software v. 13 (Stat-Ease Inc., Minneapolis, MN, USA) was used for performing the RSM and predicting the optimum extraction conditions for total phenolic and antioxidant activity from *R. tomentosa*. In order to assess the quality of the statistical model, ANOVA was also done.

All of the data in this study, both total phenolic content and percentage inhibition in the text, tables, and figures, are presented as the mean±SD.

RESULTS AND DISCUSSION

Screening of NADES for the extraction of total phenolic compound

The result showed that different types of HBD used in NADES preparation can affect the total phenolic yields. The total phenolics level of five different choline chloride-based NADES was evaluated. Fig. 1 showed that the combination of choline chloride and propylene glycol at a molar ratio of 1:1 demonstrated the highest ($P<0.05$) yields of total phenolic compounds. Surprisingly, because these results are similar to our previous studies, which showed that this combination also provided high extraction yield and excellent bioactivity from *Ixora javanica* flowers [2, 13]. Various HBA and HBD compositions have been shown in earlier research to have an impact on the physicochemical properties including polarity of NADES as well as their extraction efficiency [20]. The viscosity of the NADES also plays an important role in their extraction efficiency [21, 22]. In this study, ChSb and ChGl showed higher vicosities than other NADES, and they provided lower extraction yields of phenolic compounds. Additionally, steric hindrance can also inhibit the formation of chemical bonds, such as hydrogen bonds. This can explain the lower yield of phenolic compounds by ChPeg compared to other solvents due to the complexity of its structure.

Moreover, not only the type of HBA and HBD, but the molar ratio also affects NADES extraction capability. Our result showed that the phenolic compound yields decreased due to increasing the propylene glycol molar ratio. The formation of hydrogen bonds decreased with increasing levels of propylene glycol and decreasing levels of choline chloride. On the other hand, the primary process in NADES that increases extraction efficiency is hydrogen bond formation [23]. Thus, combination of choline chloride and propylene glycol with molar ratio of 1: 1 was selected for subsequent extraction and analysis procedures.

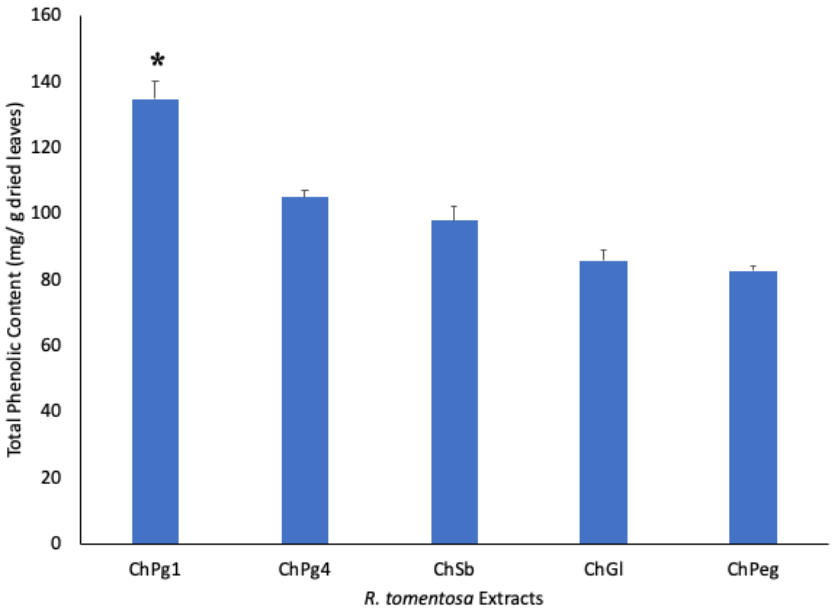


Fig. 1: Comparison of total phenolic compound yields among the different NADES data are expressed as mean±SD (n=5; *P<0.05)

Table 3: 15-run experimental responses

Run	Independent variable			Responses	
	X_1[a]	X_2[b]	X_3[c]	TPC (mg GAE/g dried leaves)	ABTS scavenging activity (%)
1	0	1	-1	11.6193	61.05
2	1	1	0	15.5615	66.94
3	-1	0	1	8.0421	52.38
4	-1	0	-1	23.3408	81.34
5	0	-1	-1	12.2224	61.99
6	-1	-1	0	5.9471	43.65
7	0	-1	1	5.5945	44.37
8	0	0	0	12.9797	63.56
9	-1	1	0	3.7645	44.24
10	0	0	0	12.9199	62.56
11	0	1	1	0.5316	40.50
12	1	-1	0	19.1300	72.45
13	1	0	1	26.6502	91.22
14	0	0	0	12.9657	62.56
15	1	0	-1	29.6351	96.84

[a]X_1 is extraction Time (min); [b] X_2 is water content in NADES (%); [c] X_3 Solid-to-liquid ratio (g/ml)

Optimization UAE condition using RSM

The independent variables optimized in this study were extraction time, water content, and solid-to-liquid ratio, while the responses were total phenolic yields and antioxidant activity from *R. tomentosa* leaves. The responses obtained from experiments in the laboratory for each combination are presented in table 3. Then, using Design Expert software, all of the responses were examined to create a regression equation-a model that predicts each response that represented in table 4.

The results are also represented as response surface plot on 3D surface graphs (fig. 2 and 3**).** Extraction time is closely related to the chance of contact between the solvent and the sample. Along with increasing contact time, the opportunity for substance diffusion will also increase. Thus, the extraction yields also increase which is in agreement with previous findings conducted by [24]. As mentioned above, viscosity becomes the common problem when applying NADES as solvent extraction. Several studies have added water to the NADES mixture to solve the viscosity problem [25, 26]. However, the addition of excess water can weaken the hydrogen bonds formed between the solvent and the sample and can even cause a change in the polarity of the NADES [27, 28]. The ratio between plant materials and volume of the solvents is known as the solid-to-liquid ratio. The compound solubility increased when the solid-to-liquid ratio is lower due to larger volume of extraction solvents. Whereas, when the solvent volume greater than the required amount, more impurities were dissolved out and prevented the dissolution of the compound. A similar result was also shown in previous work conducted by Jing *et al.* [29].

Table 4: Regression model

Response	Model equation
Total phenolic content (mg GAE/g dried leaves)	$y = 12.96 + 6.24x_1 - 1{,}43x_2 - 4.50x_3 - 0.3465x_1x_2 + 3.08x_1x_3 - 1.11x_2x_3 + 6.29x_1^2 - 8.14x_2^2 + 2.68x_3^2$
ABTS radical scavenging activity (%)	$y = 62.89 + 13.23x_1 - 1{,}22x_2 - 9.09x_3 - 2.53x_1x_2 + 5.84x_1x_3 - 0.7316x_2x_3 + 11.20x_1^2 - 17.27x_2^2 + 6.36x_3^2$

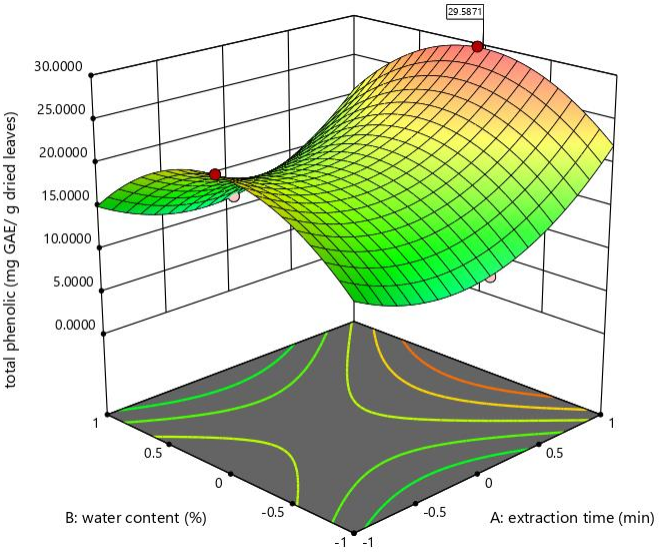
Table 5, 6 presents the results of an ANOVA that was conducted to assess the model quality. There is strong agreement between the experimental results and the predicted yield, as indicated by the R^2 values of all the models. The model can express variances of more than 99.93% (R^2 = 0.9993) and 98.94% (R^2 = 0.9894) for total phenolic yield and antioxidant activity, respectively. The result also demonstrate that the responses are significantly impacted by the variables (p-value<0.05). Based on the p-value, it can also be seen that the variables interact with each other to have an effect on the response. In addition, the models indicate that the statistical insignificance of the lack of fit (p-value>0.05), with the phenolic and antioxidant models showing p-values of 0.0660 and 0.2892, respectively. A model can be categorized as a good model if it has an insignificant lack-of-fit value that indicates the model's inability to adequately represent the data is not significant, so it is suitable to predict the response [30].

Table 5: ANOVA for total phenolic content prediction model

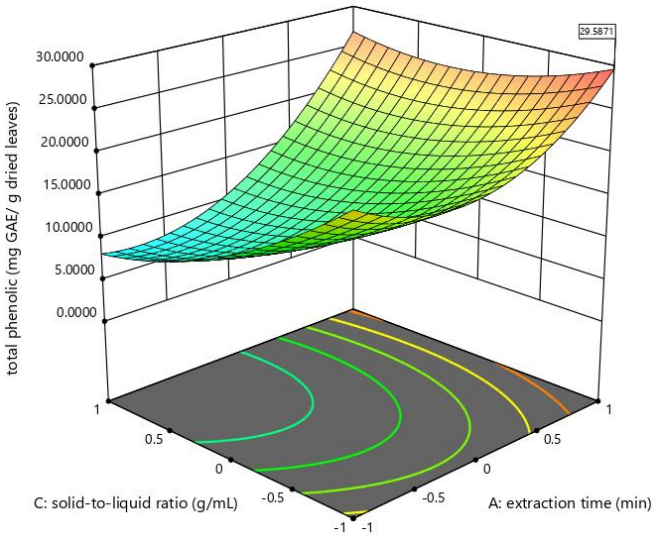
Source	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Model	984.20	9	109.36	12444.91	<0.0001
X_1	311.03	1	311.03	35396.10	<0.0001
X_2	16.29	1	16.29	1854.27	<0.0001
X_3	161.99	1	161.99	18435.16	<0.0001
X_1X_2	0.4802	1	0.4802	54.65	0.0007
X_1X_3	37.91	1	37.91	4313.98	<0.0001
X_2X_3	4.97	1	4.97	565.88	<0.0001
X_1^2	145.87	1	145.87	16600.24	<0.0001
X_2^2	244.63	1	244.63	27839.94	<0.0001
X_3^2	26.45	1	26.45	3010.27	<0.0001
Residual	0.0439	5	0.0088		
Lack of Fit	0.0420	3	0.0140	14.30	0.0660
Pure Error	0.0020	2	0.0010		
Cor total	984.24	14			

Table 6: ANOVA for ABTS radical scavenging activity prediction model

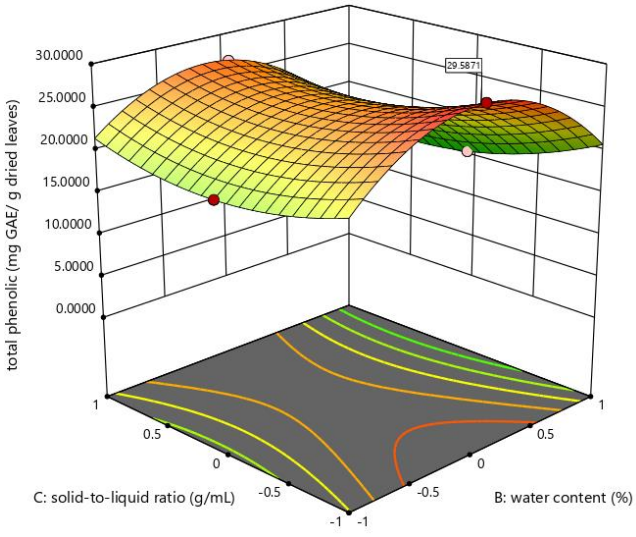
Source	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Model	4077.62	9	453.07	691.72	<0.0001
X_1	1400.40	1	1400.40	2138.05	<0.0001
X_2	11.86	1	11.86	18.10	0.0081
X_3	661.57	1	661.57	1010.05	<0.0001
X_1X_2	9.31	1	9.31	14.21	0.0130
X_1X_3	136.22	1	136.22	207.97	<0.0001
X_2X_3	2.14	1	2.14	3.27	0.1304
X_1^2	462.91	1	462.91	706.74	<0.0001
X_2^2	1101.32	1	1101.32	1681.43	<0.0001
X_3^2	149.14	1	149.14	227.70	<0.0001
Residual	3.27	5	0.6550		
Lack of Fit	2.61	3	0.8694	2.61	0.2892
Pure Error	0.6667	2	0.3333		
Cor Total	4080.90	14			



(a)

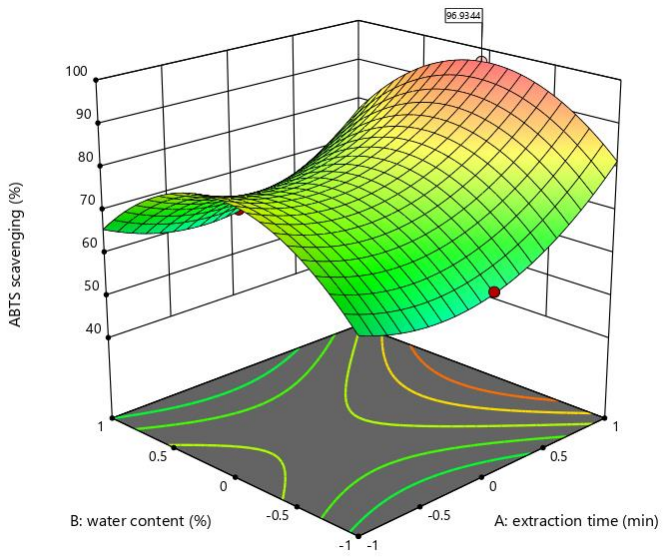


(b)

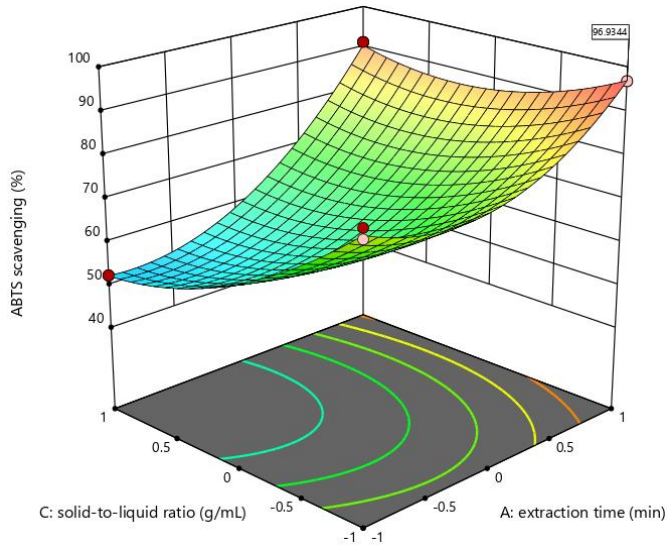


(c)

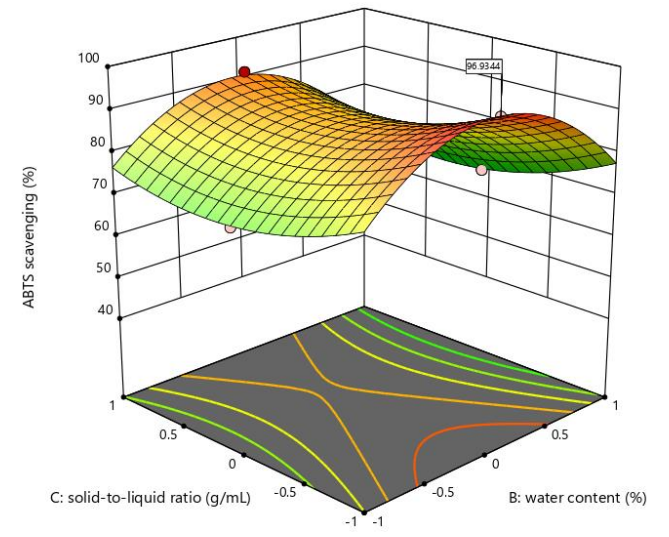
Fig. 2: 3D response surface graphs of total phenolic yield versus (a) extraction time (x_1) and water content (x_2); (b) extraction time (x_1) and solid-to-liquid ratio (x_3); (c) water content (x_2) and solid-to-liquid ratio (x_3)



(a)



(b)



(c)

Fig. 3: 3D response surface graphs of ABTS scavenging activity versus (a) extraction time (x_1) and water content (x_2); (b) extraction time (x_1) and solid-to-liquid ratio (x_3); (c) water content (x_2) and solid-to-liquid ratio (x_3)

The present study employed all the experimental results to forecast the ideal extraction conditions for enhancing total phenolic content and antioxidant activity from *R. tomentosa* leaves. The statistically analysis using RSM software showed that the ideal NADES-UAE conditions for *R. tomentosa* leaves are an extraction time of 60 min, 25% water content, and a solid-to-liquid ratio of 0.02 g/ml.

Table 7 demonstrates that the model was suitable to predict the data. This can be seen from the optimal values from experimental tests and software predictions, which are close to each other. We also succeeded in proving that our optimal extraction condition showed better total phenolic and antioxidant activity compared to an ethanolic extract of *R. tomentosa* leaves.

We tried to investigate the relationship between phenolic compounds and antioxidant activity, and the results can be seen in fig. 4. The result showed that the R^2 value between total phenolic vs ABTS scavenging is 0.986 that indicates there is a good correlation and the activity antioxidant is mediated by phenolic compounds. This result is in accordance with research conducted by Lestari *et al.* [31], where there was consistency between the levels of phenolic compounds in the samples and their antioxidant activity. This also ensures that in our recent study we succeeded in extracting maximum phenolic compounds and antioxidant activity simultaneously from *R. tomentosa* leaves.

Table 7: Value of all response variables, both experimental and predicted, under ideal UAE conditions and comparison to ethanolic extract

Response	Experimental value	Predicted value	Ethanolic extract
Total phenolic content (mg GAE/g dried leaves)	29.6351	29.6351	22.5674 ± 0.2669
ABTS scavenging activity (%)	96.84	96.93	73.12 ± 0.64

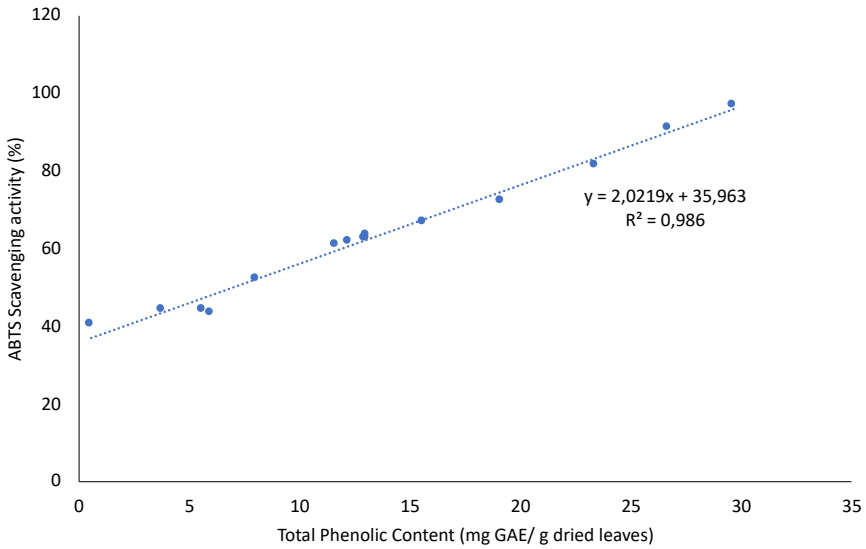


Fig. 4: Graphs represent correlation curve between total phenolic content vs antioxidant activity

CONCLUSION

A green extraction optimization-facilitated Response Surface Methodology (RSM) was successfully carried out to obtain the extraction condition of *R. tomentosa* leaves with the highest extraction yield of total phenolic compound and anti-oxidant activity simultaneously. The environmentally friendly extraction method using NADES-UAE has been successfully develop. The best extraction conditions that this study recommended were extraction time of 60 min, water content of 25%, and a solid-to-liquid ratio of 0.02 g/ml. Our finding demonstrated that using NADES consisting of choline chloride and propylene glycol at a molar ratio of 1:1, along with the UAE method, under our ideal extraction conditions, can be an acceptable alternative for conventional organic solvent extraction methods that are both more efficient and environmentally friendly.

ACKNOWLEDGEMENT

The authors thank for the financial support provided by The Directorate General of Higher Education, Research, and Technology of the Republic of Indonesia for Fundamental Programme 2023 grant ref no. 183/E5/PG.02.00. PL/2023; 004/SP2H/PT/lL7/2023; 040/SP-Lit/lPPM-01/KemendikbudRistek/FF/VI/2023.

AUTHORS CONTRIBUTIONS

NDO-Conceptualization of ideas, designed the experimental, performed the experiments, data acquisition, data analysis, statistical analysis, manuscript preparation, editing, and review; RB-performed the experiments, data acquisition, data analysis, statistical analysis, manuscript preparation; EWF-performed the experiments, data acquisition, data analysis, statistical analysis, manuscript preparation; CA-Conceptualization of ideas, designed the experimental, data curation, data analysis, statistical analysis, manuscript preparation, editing, and review.

CONFLICT OF INTERESTS

Declared none

REFERENCES

1. Lai TN, Herent MF, Quetin Leclercq J, Nguyen TB, Rogez H, Larondelle Y. Piceatannol a potent bioactive stilbene as major phenolic component in *Rhodomyrtus tomentosa*. Food Chem. 2013;138(2-3):1421-30. doi: 10.1016/j.foodchem.2012.10.125, PMID 23411263.
2. Oktaviyanti ND, Kartini MA, Mun IM A. Application and optimization of ultrasound-assisted deep eutectic solvent for the extraction of new skin lightening cosmetic materials from *Ixora javanica* flower. Heliyon. 2019;5(11):e02950. doi: 10.1016/j.heliyon.2019.e02950, PMID 31844780.
3. Apridamayanti P, Sari R, Pratiwi L. Development validation of quercetin compounds using RP-HPLC and *in vitro* activity studies on *melastoma malabathricum* leaf nanocream

foundation preparations. Int J App Pharm. 2023;5(5):317-24. doi: 10.22159/ijap.2023v15i5.48297.

4. Koraqi H, Petkoska AT, Khalid W, Sehrish A, Ambreen S, Lorenzo JM. Optimization of the extraction conditions of antioxidant phenolic compounds from strawberry fruits (Fragaria x ananassa Duch.) using response surface methodology. Food Anal Methods. 2023;16:1-13. doi: 10.1007/s12161-023-02469-6, PMID 37359894.
5. Fierascu RC, Temocico G, Fierascu I, Ortan A, Babeanu NE. Fragaria genus: chemical composition and biological activities. Molecules. 2020;25(3):498. doi: 10.3390/molecules25030498, PMID 31979351.
6. Ridlo M, Kumalaningsih S, Pranowo D. Optimization of microwave assisted extraction from rhodomyrtus tomentosa fruits using response surface methodology. IOP Conf Ser: Earth Environ Sci. 2019;230:012041. doi: 10.1088/1755-1315/230/1/012041.
7. Ismandari T, Kumalaningsih S, Wijana S, Mustaniroh SA. Optimization of bioactive compound extraction from rose myrtle fruit (rhodomyrtus tomentosa, (wait) myrtaceae) as the antioxidant source. Scientific World Journal. 2020;2020:9105847. doi: 10.1155/2020/9105847, PMID 32395089.
8. DE Carvalho CC, DA Fonseca MM. Solvent toxicity in organic aqueous systems analysed by multivariate analysis. Bioprocess Biosyst Eng. 2004;26(6):361-75. doi: 10.1007/s00449-004-0381-1, PMID 15378340.
9. Levet A, Bordes C, Clement Y, Mignon P, Morell C, Chermette H. Acute aquatic toxicity of organic solvents modeled by QSARs. J Mol Model. 2016;22(12):288. doi: 10.1007/s00894-016-3156-0, PMID 27830479.
10. Lin CH, Lai CH, Peng YP, WU PC, Chuang KY, Yen TY. Comparative health risk of inhaled exposure to organic solvents toxic metals and hexavalent chromium from the use of spray paints in Taiwan. Environ Sci Pollut Res Int. 2019;26(33):33906-16. doi: 10.1007/s11356-018-2669-8, PMID 29974442.
11. Seo S, Kim J. An aggravated return to work case of organic solvent induced chronic toxic encephalopathy. Ann Occup Environ Med. 2018;30(1):27. doi: 10.1186/s40557-018-0232-1, PMID 29719722.
12. Oktaviyanti ND, Kartini K, Hadiyat MA, Rachmawati E, Wijaya AC, Hayun H. A green extraction design for enhancing flavonoid compounds from *Ixora javanica* flowers using a deep eutectic solvent. R Soc Open Sci. 2020;7(10):201116. doi: 10.1098/rsos.201116, PMID 33204470.
13. Oktaviyanti ND, Setiawan F, Kartini K, Azminah A, Avanti C, Hayun H. Development of a simple and rapid HPLC-UV method for ultrasound assisted deep eutectic solvent extraction optimization of ferulic acid and antioxidant activity from *ixora javanica* flowers. S Afr J Chem Eng. 2022;40:165-75. doi: 10.1016/j.sajce.2022.03.004.
14. Jeong KM, KO J, Zhao J, Jin Y, Yoo DE, Han SY. Multi functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products. J Clean Prod. 2017;151:87-95. doi: 10.1016/j.jclepro.2017.03.038.
15. Rizkiyan Y, Suharyani I, Falya Y, Amelia R, Nuh M, Sulastri L. Nades extract of gedong mango leaves and mulberry leaves in spray gel as a sunscreen. Int J App Pharm. 2022;14(4):121-5. doi: 10.22159/ijap.2022.v14s4.PP29.
16. Pena Pereira F, Tobiszewski M. The application of green solvent in separation process. Amsterdam: Elsevier; 2017.
17. Jing CL, Dong XF, Tong JM. Optimization of ultrasonic-assisted extraction of flavonoid compounds and antioxidants from alfalfa using response surface method. Molecules. 2015;20(9):15550-71. doi: 10.3390/molecules200915550, PMID 26343617.
18. Aleixandre Tudo JL, Buica A, Nieuwoudt HH, Aleixandre JL, DU Toit W. Spectrophotometric analysis of phenolic compounds in grapes and wines. J Agric Food Chem. 2017;65(20):4009-26. doi: 10.1021/acs.jafc.7b01724, PMID 28475326.
19. Jacob J, Lakshmanapermalsamy P, Illuri R, Bhosle D, Sangli GK, Mundkinajeddu D. *In vitro* evaluation of antioxidant potential of isolated compounds and various extracts of peel of *Punica granatum* L. Pharmacognosy Res. 2018;10(1):44-8. doi: 10.4103/pr.pr_36_17, PMID 29568186.
20. Zainal abidin MH, Hayyan M, Hayyan A, Jayakumar NS. New horizons in the extraction of bioactive compounds using deep eutectic solvents: a review. Anal Chim Acta. 2017;979:1-23. doi: 10.1016/j.aca.2017.05.012, PMID 28599704.
21. Dai Y, Rozema E, Verpoorte R, Choi YH. Application of natural deep eutectic solvents to the extraction of anthocyanins from *Catharanthus roseus* with high extractability and stability replacing conventional organic solvents. J Chromatogr A. 2016;1434:50-6. doi: 10.1016/j.chroma.2016.01.037, PMID 26822320.
22. LI Z, Lee PI. Investigation on drug solubility enhancement using deep eutectic solvents and their derivatives. Int J Pharm. 2016;505(1-2):283-8. doi: 10.1016/j.ijpharm.2016.04.018, PMID 27079143.
23. Sang J, LI B, Huang Y, MA Q, Liu K, Li C. Deep eutectic solvent based extraction coupled with green two dimensional HPLC-DAD-ESI-MS/MS for the determination of anthocyanins from lycium ruthenicum murr fruit. Anal Methods. 2018;10(10):1247-57. doi: 10.1039/C8AY00101D.
24. Selahvarzi A, Ramezan Y, Sanjabi MR, Namdar B, Akbarmivehie M, Mirsaeedghazi H. Optimization of ultrasonic assisted extraction of phenolic compounds from pomegranate and orange peels and their antioxidant activity in a functional drink. Food Biosci. 2022;49:101918. doi: 10.1016/j.fbio.2022.101918.
25. Cao J, Wang H, Zhang W, Cao F, MA G, SU E. Tailor made deep eutectic solvents for simultaneous extraction of five aromatic acids from *Ginkgo biloba* leaves. Molecules. 2018;23(12):3214. doi: 10.3390/molecules23123214, PMID 30563161.
26. Makos P, Slupek E, Gębicki J. Hydrophobic deep eutectic solvents in microextraction techniques a review. Microchem J. 2020;152:104384. doi: 10.1016/j.microc.2019.104384.
27. Bosiljkov T, Dujmic F, Cvjetko Bubalo M, Hribar J, Vidrih R, Brncic M. Natural deep eutectic solvents and ultrasound assisted extraction: green approaches for extraction of wine lees anthocyanins. Food Bioprod Process. 2017;102:195-203. doi: 10.1016/j.fbp.2016.12.005.
28. Hammond OS, Bowron DT, Edler KJ. The effect of water upon deep eutectic solvent nanostructure: an unusual transition from ionic mixture to aqueous solution. Angew Chem Int Ed Engl. 2017;56(33):9782-5. doi: 10.1002/anie.201702486, PMID 28480595.
29. Jing CL, Dong XF, Tong JM. Optimization of ultrasonic assisted extraction of flavonoid compounds and antioxidants from alfalfa using response surface method. Molecules. 2015;20(9):15550-71. doi: 10.3390/molecules200915550, PMID 26343617.
30. Liang CP, Chang CH, Liang CC, Hung KY, Hsieh CW. *In vitro* antioxidant activities free radical scavenging capacity and tyrosinase inhibitory of flavonoid compounds and ferulic acid from spiranthes sinensis (Pers.) Ames. Molecules. 2014;19(4):4681-94. doi: 10.3390/molecules19044681, PMID 24739930.
31. Lestari U, Muhaimin M, Chaerunisaa AY, Sujarwo W. Antioxidant activities and phytochemical screening of ethanol extract from surian leaves (*Toona sinensis*). Int J App Pharm. 2023;15(2):37-43. doi: 10.22159/ijap.2023.v15s2.07.