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Study on Optimum Extraction Conditions for Galantamine from *Galanthus woronowii L. Bulbs*

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Authors' contributions

This work was carried out in collaboration between all authors. Author IB designed the study, performed the statistical analysis. Author SG performed HPLC analysis and managed the literature searches. Author HE wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

In the present study, an ultrasound-assisted extraction (UAE) method was developed for the efficient extraction of Galantamine from the bulbs of *Galanthus woronowii L.* Five independent variables, including pH of the extraction solvent, solvent/material ratio, ultrasound time, ultrasound temperature and ultrasound power were studied by single factor experiments. The central composite design and response surface methodology were employed to investigate the effect of three key parameters (ultrasound time, ultrasound temperature and solvent/material ratio) on the extraction efficiency. The 3-level, 3-factorial Central-Composite Design was employed to study three main extraction conditions: extraction time (15-45 min), extraction temperature (30–70°C) and solvent/material ratio (30–50 mL/g sample). The present analysis revealed that a quadratic polynomial model can be used to express the response dependent variable yield of Galantamine.

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The optimal extraction conditions were found to be solvent/material ratio of 40.70 mL/g sample, with an extraction time of 32.89 min and a temperature of 51.04°C. Theoretical optimal yield was recorded 0.469% with a mentioned extraction conditions and the yield of Galantamine was found to be 0.470% which is in a very good agreement with the theoretically predicted one.

Keywords: Galantamine; Galanthus woronowii; extraction; central-composite design.

1. INTRODUCTION

The family of Amaryllidaceae consists of about 85 genera and 1100 species,distributed mainly in warm and tropical regions around the world [1]. Among these the genus *Galanthus* is represented by 14 taxa and one hybrid in Turkey [2]. Previous phytochemical investigations on *Galanthus woronowii L.* resulted in the isolation of several Amaryllidaceae alkaloids namely galantamine, lycorine, galanthine, demethylhomolycorine, galanthusine and tazettine [3,4].

G. woronowii L., known as ‘‘Karadeniz kardeleni’’ among local people, is commonly found around the southeastern Black Sea coast area. In Turkey, it is distributed in the northeastern coast [2]. This Amaryllidaceae family has long been used by researchers because of the alkaloid and particularly galantamine content of many of its members. Galantamine is a tertiary amine and belongs to the isoquinoline alkaloids class which is derived from tyrosine [5]. It is an inhibitor of acetyl cholinesterase, an enzyme that hydrolyses the neurotransmitter acetylcholine. It is an allosteric modulator of the neuronal nicotinic receptor for acetylcholine [5]. As an AChE inhibitor, approved in Europe by the European registration bureau and in the USA by FDA for the symptomatic treatment of Alzheimer’s disease, Galantamine is now commercially available as Razadyne, Beklamen and Reminyl [6]. AChE inhibitors are used to treat Alzheimer’s disease [6]. As stated in [5], since 1 out of 10 people above 65 years-old and almost half those older than 85 suffered from Alzheimer, it is imperative to find new cheap natural sources of galantamine and to develop improved extraction and purification methods. Recently, Galantamine is mainly produced synthetically by using a patented method that involves several steps [7]. Hille et al. [8] patented a liquid–liquid extraction method followed by subsequent acid–base purification steps with diethyl ether to obtain galantamine. Another company, Agroceuticals Products (Wales, UK) claims that they can obtain natural Galantamine from daffodils but have not revealed their extraction process so far. Supercritical fluid extraction is a modern technology with an increasing number of applications in the pharmaceutical and food processing industry. Galantamine is a basic alkaloid, present as a salt in plants. Great amount of effort was put into finding the appropriate basic pre-treatment conditions since these vary according to the alkaloid. The molecular structure of Galantamine and *Galanthus woronowii L.* are shown in Fig. 1.

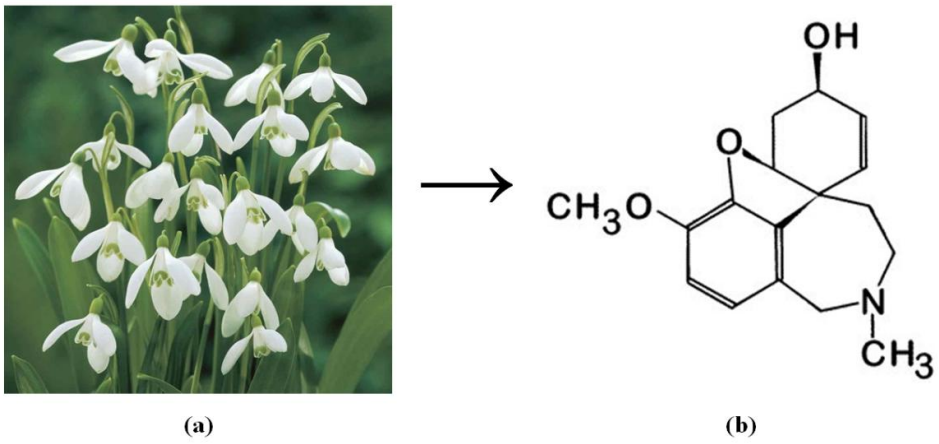

Fig. 1. (a). *Galanthus woronowii L.* (b). Molecular structure of galantamine

Extraction is the first important step in the recovery and purification of active ingredients of plant materials. Improvement in the extraction based on energy and time, along with obtaining a higher bioactivity has led to adoption of newer techniques such as microwave assisted extraction (MAE), supercritical fluid extraction (SFE), accelerated solvent extraction (ASE) and ultrasound assisted extraction (UAE) [9, 10]. Among these methods, UAE is considered as a cost-effective technique based on its short extraction time and reduced power consumption attributed to the physiochemical effects of cavitation phenomenon induced with ultrasound waves into the extraction medium [11,12]. However, the successful application of UAE depends relatively on the optimum operating conditions and the matrix of the plants subjected to ultrasound [13], which should be investigated carefully towards obtaining a higher yield of targeted components and their bioactivity. The operational parameters such as power intensity, irradiation time and temperature could be optimised significantly to obtain the best combination of their interacted levels for better influence on the concentration, stiffness and matrix of the extracted raw materials. Actually, one of the different approaches that illustrate the effects of power intensity on the ultrasound assisted process was endorsed by Patist and Bates [11]. According to Patist and Bates, amplitude, pressure, temperature, viscosity and concentration of solids can affect the ultrasonic liquid processing. If the pressure, viscosity of the solvent and the concentration of the solid materials were considered constant, the optimal effects of the remaining parameters could be a function of energy (the total of energy input per volume of treated materials, kWh/L) and intensity (the actual output power per surface area of the sonotrode, W/cm^2) [11]. For a fixed tip diameter, the total energy input into the extraction medium is actually the result of power output (kW) dissipated across the cross-sectional area of tip and time of exposure of the materials subjected to ultrasound. Therefore, the optimal power intensity could be the best functioning for larger scales depends upon the capacity of the extraction, i.e., the size of the extraction vessel, as well as the geometry of the extractor.

A statistical analysis technique, Response surface methodology (RSM), was employed to systematically optimise the ultrasonic parameters involved in the extraction and to achieve their best possible combination.. It consists of a number of statistical designs such as Central Composite Design (CCD), Box-Behnken Design (BBD), Optimal Design and other statistical procedures. within the designs mentioned above, BBD has been distinguished as a simplified design to cover three levels of experimental factors with less number of experiments [14-16].

As UAE combines the advantages of short time, less solvent requirement, and low-temperature operation, the main aim of this study is to develop a facile UAE protocol for extraction of galantamine from the bulbs of *Galanthus woronowii L. and* to optimise its conditions by RSM. In the present investigation, RSM-CCD was employed to optimize the effects of ultrasound time, extraction temperature and solvent/material ratio on the yield of Galantamine extracted from the bulbs of *Galanthus woronowii L.* The optimal conditions achieved in this study have been applied to scale-up the UAE of Galantamine.

2. MATERİALS AND METHODS

The plant sample was collected from Trabzon, Turkey. Its bulbs were rendered and dried at 80°C in an oven. Dried bulbs were ground into of 80-100 mesh size before extraction.

All chemicals used for conducting the experiments were of analytical grade and all solvents used for chromatographic purposes were of HPLC grade. Membranes (0.45 µm) (Millipore, Bedford, MA, USA) were used for filtering all the solutions. Galantamine standard was purchased from Sigma Chemical Co.

The ultrasound-assisted extraction was carried out in a Wisebath brand ultrasonic water bath with an electric power of 900 W, and 50 kHz frequency, equipped with a digital timer and a temperature controller to control both time and temperature. The bath consisted of a rectangular vessel (20.2 cm X 17.5 cm X 22.9 cm). The ultrasonic energy was delivered from bottom to the water with a relatively constant frequency of 50 kHz.

2.1 UAE Extraction Procedure

Precisely 1.0 g of dried and ground sample was placed in a round bottom flask. 40 ml of 0.1 M HCl was added in it. For the standard ultrasonic conditions, Erlenmeyer flasks were placed inside the ultrasonic bath. The solvent level in the Erlenmeyer flask and the water level in the ultrasonic bath were kept the same. The

Table 1. Variables and their five levels employed in the central composite design

Independent variables	Units	Symbols	Code levels				
			-1.68	-1	0	1	1.68
Ext. time	min	(X_1)	4.80	15	30	45	55.20
Ext. temp.	˚C	(X_2)	16.36	30	50	70	83.64
Solvent/Material ratio	mL/g Sample	(X_3)	23.18	30	40	50	56.83

temperature and time value of the ultrasonic bath was set and extraction was carried out. After the extraction procedure, firstly the bulbs extracts were filtered through Whatman filter paper and then filtered with 0.45 micron membrane filter (Millipore, Bedford, MA, USA).

2.2 Quantification of GA by High-Performance Liquid Chromatography

HPLC analysis of galantamine was established by Agilent 1260 chromatographic system equipped with autosampler, quaternary pump, column compartment and a UV-VIS detector system. A reverse phase ACE brand C18 Column (250 x 4.6 mm. 5 µm.) was used as the stationary phase at 30°C. UV detection was done at 290 nm. The mobile phase assayed was 0.05 M potassium dihydrogen phosphate:acetonitrile in the ratio of 90:10 (v/v). The mobile phase was filtered through 0.45 µm Millipore filters. The flow rate was 1.0 mL/min and the injection volume was 10 µL. The retention time of galantamine was 5.8 minutes. The calibration curve was linear ((Y = 4.6863 X + 4,894)(R^2 = 0.9999)) in the concentration range of 100-500 ppm. The method was statistically validated for precision, accuracy, LOD, LOQ, robustness and recovery.

2.3 Single-Factor Experiments

To evaluate the effect of each factor under ultrasound treatment on the extraction efficiency, pH (1, 4, 7, 10, 13), solvent/material ratio (25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1 mL/g), ultrasound time (0, 10, 20, 30, 40, 50, 60, 70 min), ultrasound temperature (30, 40, 50, 60, 70, 80˚C) and ultrasound power (300, 400, 500, 600, 700, 800 W) were investigated as single factor variables in the experimental design. Three variables that significantly affected the extraction efficiencies were selected for the subsequent experiments.

2.4 Response Surface Methodology Experiments

Response surface method was used to find the optimal condition of ultrasound-assisted extraction. According to the results of single factor optimisation, three variables were selected. Each variable was coded as X_1–X_3 and examined in five levels (Table 1). The 20 experimental runs including six replicates at the center point were employed. The Design-Expert (DE) design assumed that the main effects of variables have interactions and are based on a second-order polynomial model [17-18], as follows:

$$Y = \beta_0 \pm \sum \beta_i X_i \pm \sum \beta_{ii} X_i^2 \pm \sum \beta_{ij} X_i X_j \quad (1)$$

Where, Y is the response value; $ß_0$ is the constant; $ß_i$ is the linear regression coefficient; $ß_{ii}$ is the quadratic regression coefficient; ßij is the interaction regression coefficient; X_i and X_j are the independent variables.

2.5 Statistical Analysis

All the experiments were performed in triplicate, and the average value ± SD (Standard deviation) was reported. Statistical analyses was performed using Design Expert 8.06.1, and Excel 2007.

3. RESULTS AND DİSCUSSİON

3.1 Single Factor Experiment

In the preliminary study, the influence of several factors (The pH of the extraction solvent, solvent/material ratio, ultrasound time, temperature and power) on the extraction efficiency was detected and analysed.

3.2 Investigation of the Effect of pH on the Extraction Efficiency

The effect of pH on the extraction efficiency was investigated by keeping the other extraction conditions constant. The pH of solvent extraction has a large effect on the efficiency of the extraction. Other extraction conditions were kept constant at S/M ratio; 40 mL/g, the ultrasound time: 30 minutes, the ultrasound temperature: 50°C and the ultrasound power: 600 W. The pH of the solvent was changed to 1, 4, 7, 10 and 13.

The effect of different solvent pH on the extraction efficiency was investigated. The results have been shown in Fig. 2. As the pH value increased, the yield decreased continuously. The highest Galantamine yield (0.464%) obtained with the extraction solvent having a pH of 1. A 0.1 M. HCl solution (pH:1) was used as the extraction solvent in subsequent extraction processes.

3.3 Investigation of the Effect of Solvent/Material Ratio on the Extraction Efficiency

To study the effect of different liquid-solid ratios on the extraction efficiency, different solvent/material ratios (25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1) were employed, pH was 1, ultrasonication time was 30 minutes, ultrasonication temperature was 50°C, and ultrasonication power was 600 W. The results are displayed in Fig. 3. When the ratio of solvent to material increased from 25:1 to 40:1, the extraction efficiency increased from 0.364 to 0.462%. When the solvent/material ratio exceeded 40:1 (mL/g), the extraction efficiency almost did not change. The reason was that the higher ratio of solvent to material might accelerate mass transfer and facilitate the diffusion of galantamine into the medium until the mass transfer process reached its maximum. Therefore, 40:1 was selected as the optimal solvent/material ratio in subsequent extraction processes.

3.4 Investigation of the Effect of Ultrasound Time on the Extraction Efficiency

The effect of different ultrasound times on the extraction efficiency was compared, and the results are shown in Fig. 4. Other extraction conditions were set as follows: pH, 1; ratio of solvent to material, 40 mL/g; ultrasound

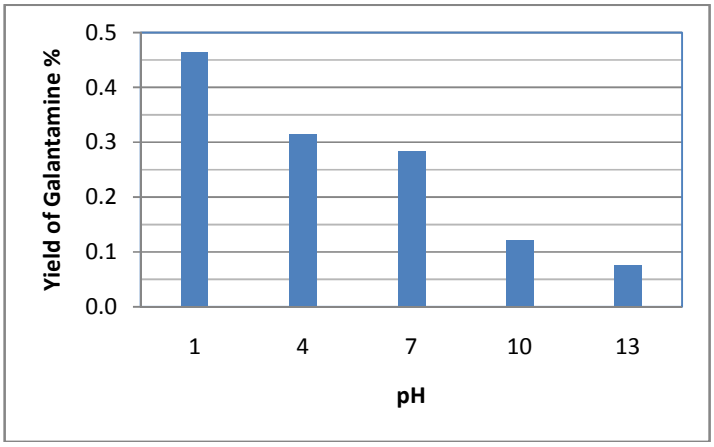

Fig. 2. The Effect of pH on the extraction efficiency

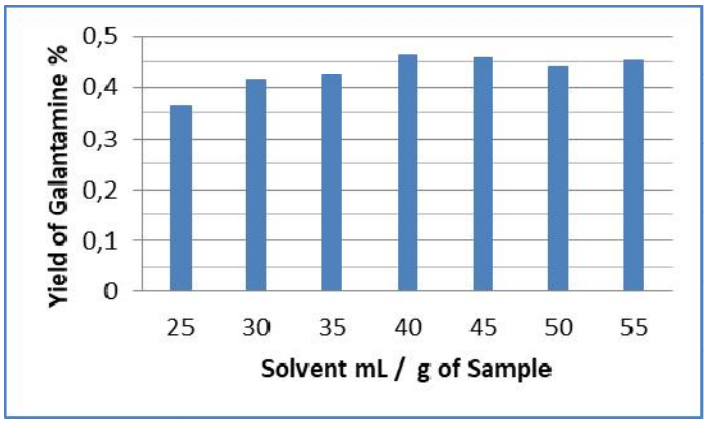

Fig. 3. The Effect of solvent/material ratio on the extraction efficiency

irradiation temperature, 50°C. The extraction yield increased from 10 to 30 min, and then decreased when the ultrasound irradiation time was longer than 30 min. The maximum extraction yield was obtained at 30 min. The results indicated that under the ultrasound treatment, the diffusion of the bioactive compounds from material to solvent might be improved and the equilibrium for dissolution might be established in a short time. But galantamine might be degraded after a long exposure to ultrasonic irradiation. Thus, 30 min ultrasound time was used in the subsequent experiments.

3.5 Investigation of the effect of ultrasound temperature on the extraction efficiency

The effect of temperature on the extraction yield was evaluated, and the results are shown in Fig. 5. Other extraction conditions were set as follows: pH, 1; solvent/material ratio, 40 mL/g; ultrasound time, 30 min. The extraction yield improved when the temperature was raised from 30 to 50°C, while the extraction yield decreased with a rise in temperature from 50 to 80°C. The maximum extraction yield (0.469%) might be obtained at 50°C. The results indicated that natural galantamine reached an equilibrium of desorption and solubility at 50°C, and the part of galantamine could be decomposed at a higher temperature. Therefore, 50°C was used in the subsequent extraction processes.

3.6 Investigation of the Effect of Ultrasound Power on the Extraction Efficiency

The ultrasound power is another essential parameter that influences the extraction. Higher ultrasound power leads to the formation and collapse of more bubbles. Ultrasound waves with a larger amplitude travel through extracting solution, so, the increase of ultrasound power

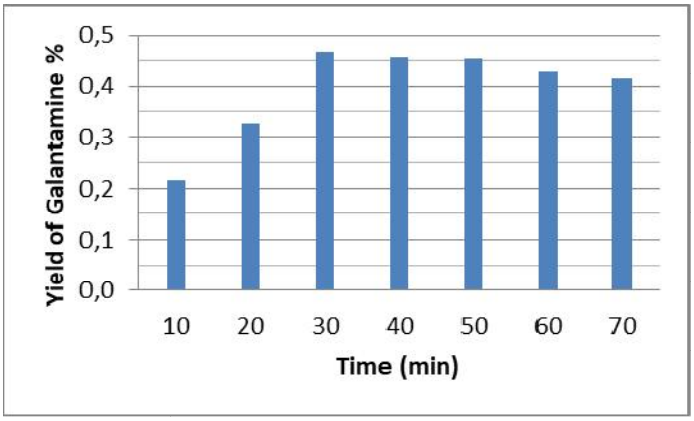

Fig. 4. The Effect of ultrasound time on the extraction efficiency

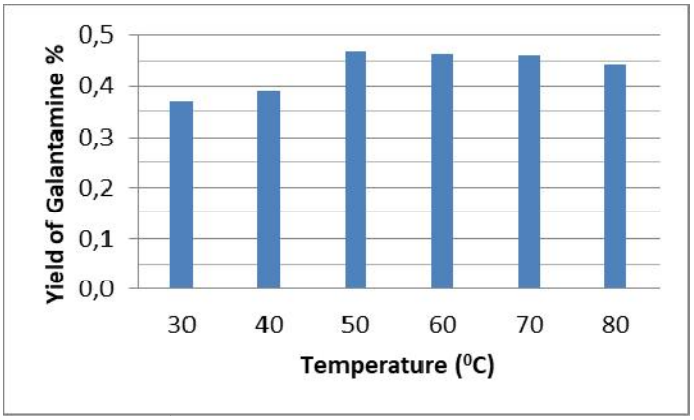

Fig. 5. The Effect of ultrasonication temperature on the extraction efficiency

might increase the yield of extraction [19,20]. However, an excessively high ultrasound power could degrade or decompose galantamine in the extracts, so the optimal ultrasound power should be investigated. A number of preliminary experiments covering the ultrasonic power levels of 300, 400, 500, 600, 700 and 800 W were conducted to check the efficiency of extraction by keeping other parameters being the same as in the single-factor pH test. As presented in Fig. 6, the extraction efficiency grew slightly with the increase of ultrasonication power primarily, and when the ultrasound power was 600 W, the highest galantamine content of the extracts was recorded (0.462%). However, the extraction efficiency showed a decreasing trend when the ultrasonication power enhanced from 600 W to 800 W. Thus, 600 W was selected as the preferred ultrasound power for UAE process.

3.7 Analytical Method Validation

The method has been validated regarding precision, accuracy, LOD, LOQ, robustness and recovery according to ICH guidelines, taking into account the recommendations of other appropriate guidelines. Results obtained from testing different parameters during the validation of the analytical method are displayed in Table 2.

3.7.1 Standard solutions and calibration curves

The standard stock solution of galantamine hydrobromide was prepared in water at the final concentration of 1000 µg.ml^{-1}. Before calibration, the stock solution was diluted with water. The standard curve was prepared over a concentration range of 100-500 µg.ml^{-1} for galantamine with five different concentration levels. Linearity for galantamine was plotted using linear regression of the peak area versus concentration. The coefficient of correlation (R2) was used to judge the linearity. The limits of detection (LOD) and quantification (LOQ) for tested compounds were determined by the signal to noise (S/N) ratio. Results obtained from testing different parameters during the validation of the analytical method have been shown in Table 2. Fig. 7 exhibits chromatogram of galantamine standard solution. Fig. 8 represents the chromatogram of the bulbs extract of *Galanthus woronowii L.*

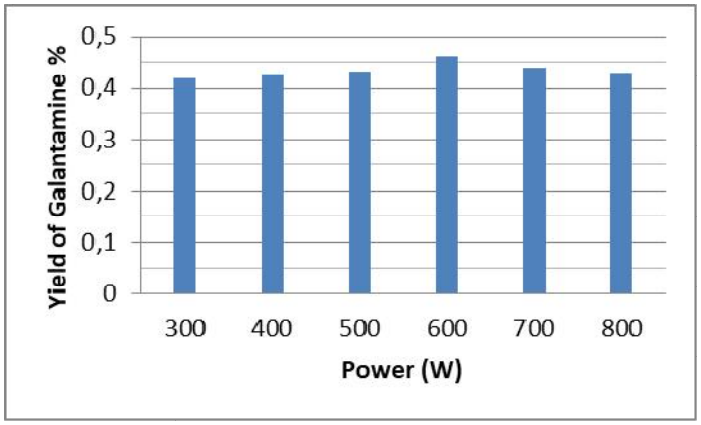

Fig. 6. The Effect of ultrasound power on the extraction efficiency

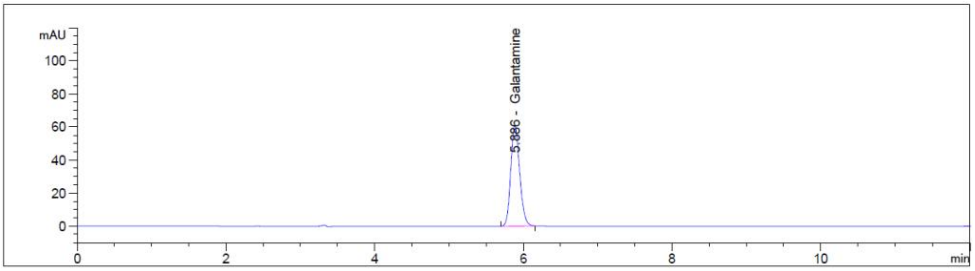

Fig. 7. The HPLC Chromatogram of Galantamine Standard

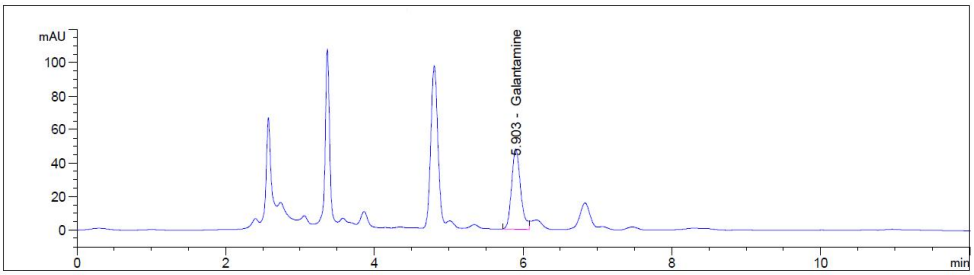

Fig. 8. The HPLC Chromatogram of Extract of *G. woronowii L.* bulbs

Table 2. Results obtained from testing different parameters during the validation of the analytical method

Parameters		Results
Specifity	Peak purity ratio	0.0010
Linearity	Concentration range µg/mL	100-500
	Correlation coefficient	0.9999
	Intercept	4.8940
	Slope	4,6863
LOD (ppm)		1.25
LOQ (ppm)		2.86
Retention Time (min.)		5.80

3.8 Response Surface Methodology

Response surface methodology was used to evaluate the interaction of several experimental parameters. .

3.9 Experimental Design and Results of CCD

According to the single factor experimental results, ultrasound time of 30 min, ultrasound temperature of 50°C and solvent/material ratio of 40:1 (mL/g) were chosen as the central condition of the central composite design and the effects of three independent variables on the dependent variable (Galantamine Content) at five levels were investigated. The 20 experimental designs and the results were shown in Table 3. The results indicated that the galantamine contents ranged from 0.324 to 0.485%. The maximum galantamine content was recorded under the experimental parameters of ultrasound time of 30 min, ultrasound temperature of 50°C and solvent/material ratio of 40 mL/g sample.

Table 3. Experimental design of response surface analysis and its experimental values

Run	Ext. time min. X_1	Ext. temp. ^0C X_2	Solvent/Material ratio mL/g sample X_3	Galantamine content % actual
1	15	30	30	0.383
2	15	30	50	0.371
3	15	70	30	0.324
4	15	70	50	0.343
5	15	30	30	0.364
6	45	30	50	0.350
7	45	70	30	0.376
8	45	70	50	0.402
9	45	50	50	0.366
10	30	50	30	0.364
11	30	70	40	0.364
12	30	30	40	0.361
13	30	50	40	0.453
14	45	50	40	0.421
15	15	50	40	0.421
16	30	50	40	0.485
17	30	50	40	0.471
18	30	50	40	0.464
19	30	50	40	0.464
20	30	50	40	0.471

3.9.1 Fitting the model

Analysis of variance (ANOVA) was performed to evaluate the quality of the fitted model (Table 4). It was revealed that, the second-order polynomial model for the extraction of galantamine was statistically significant with a small model p-value ($p < 0.0001$) and satisfactory coefficient of determination (R2 = 0.9558). The linear (X_2, X_3) and quadratic parameters (X_2^2 and X_3^2) were significant at a level of $p < 0.01$, interaction parameters (X_1X_2) and quadratic parameter (X_1^2) were significant at a level of $p < 0.05$. The "Lack of Fit-Value" of the model is not significant with a p-value of 0.1211. The significant regression and non-significant lack of fit indicated that the regression equation is adequate to represent the actual relationship between the response values and three independent variables. The quadratic regression equation was obtained as follows [Equation (2)]:

$$G\,\% = -0{,}35870 + 0.002493 \times X_1 + 0.007385 \times X_2 + 0{,}029398 \times X_3 + 0.000058 \times X_1 \times X_2 + 0.000008 \times X_1 \times X_3 + 0.000029 \times X_2 \times X_3 - 0.000088 \times {X_1}^2 - 0.000103 \times {X_2}^2 - 0.000383 \times {X_3}^2 \quad (2)$$

3.9.2 Analysis of response surfaces

The response surface plots are given in Fig. 9. The interaction between various factors can be seen directly from the response surfaces plots. Fig. 9A shows the effect of the interaction of extraction temperature and solvent/material ratio on the extraction efficiency at a fixed ultrasound irradiation time of 33 min. An increase of liquid-to-solid ratio (X_3) resulted in an increase of extraction yield to a maximum limit at a certain level, while a rise in extraction temperature (X_2) resulted in an initial increase of extraction efficiency and then decreased as the temperature continued to increase. Fig. 9B shows the effect of interaction of solvent/material ratio and ultrasound time on the extraction efficiency at a fixed extraction temperature of 51°C. It could be observed that the solvent/material ratio (Fig. 9B) resulted in similar effects on the extraction efficiency as in Fig. 9A, whereas ultrasound irradiation time had only a limited impact on the extraction efficiency. Fig. 9C shows the effect of interaction of ultrasound temperature and ultrasound time on the extraction efficiency at a fixed solvent/material ratio of 40.66. It could be observed that the ultrasound temperature demonstrated a strongly positive influence on the extraction efficiency, whereas ultrasound time had only a slight impact. The combination of analysis of variance (ANOVA) (Table 3) and response surfaces (Fig. 9) indicated that the interaction effect between ultrasound time and ultrasound temperature (X_1X_2) was statistically significant, whereas the interaction effect between ultrasound time and solvent/material ratio (X_1X_3), and ultrasound temperature and solvent/material ratio (X_2X_3) were non-significant. Furthermore, it could be concluded that the effect of ultrasound temperature and solvent/material ratio were more significant than ultrasound time on the extraction efficiency.

Table 4. ANOVA for the response surface quadratic model

Source	Sum of squares	dF	Mean square	F value	p value	Significance
Model	0,047000	9	0.005201	24.050	< 0.0001	significant
X_1	0.000293	1	0.000293	1.350	0.2718	not significant
X_2	0.005097	1	0.005097	23.570	0.0007	significant
X_3	0,015000	1	0,015000	70.870	< 0.0001	significant
X_1X_2	0.001888	1	0.001888	8.730	0.0144	significant
X_1X_3	0.000012	1	0.000012	0.053	0.8220	not significant
X_2X_3	0.000214	1	0.000214	0.990	0.3429	not significant
X_1^2	0.005302	1	0.005302	24.510	0.0006	Significant
X_2^2	0.024000	1	0.024000	109.600	< 0.0001	Significant
X_3^2	0.018000	1	0.018000	84.470	< 0.0001	Significant
Residual	0.002163	10	0.000216			
Lack of Fit	0.001418	4	0.000355	2.860	0.1211	not significant
Pure Error	0.000745	6	0.000124			
Cor Total	0.049000	19				
R-Squared	0.9558					
Adj R-Squared	0.9161					

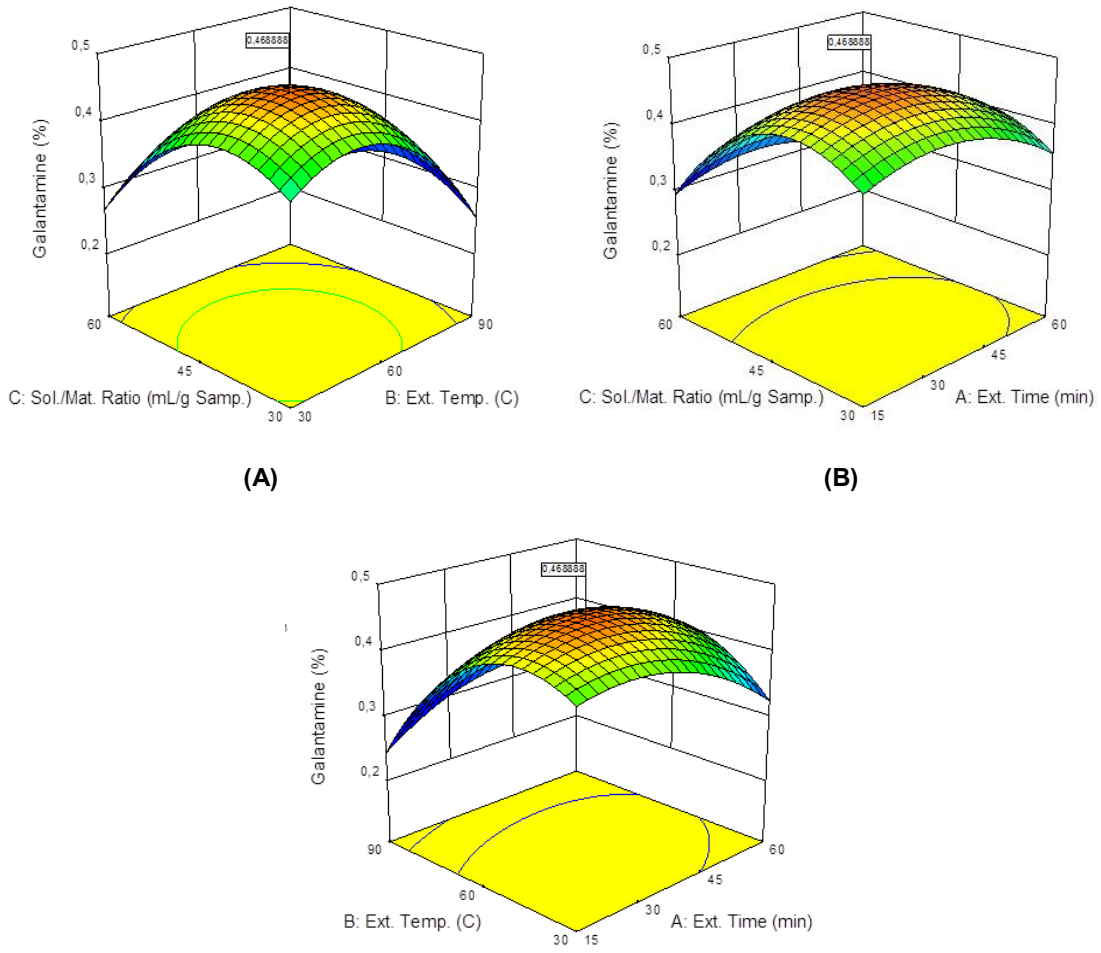

Fig. 9. Interaction effects of solvent/material ratio and ultrasound temperature (A); solvent/material ratio and ultrasound time (B); ultrasound temperature and ultrasound time (C) on the extraction efficiency

3.9.3 Verification of predicted value of the models

The optimal conditions obtained using the model was recorded as follows: extraction time, 32.89 min and temperature, 51.04°C, and solvent/material ratio, 40.70 mL/g sample. Under the optimal conditions, the maximum response value of 0.469% was predicted by the model. Verification experiments were performed at the predicted conditions. The result showed that the experimental value (0.470%; n = 6) was consistent with the predictive value (Table 5). The good correlation between predicted and experimental value demonstrated that response surface methodology is accurate and reliable to find the optimum ultrasound extraction conditions for galantamine from the bulbs of *G. woronowii L.*

Table 5. Optimum conditions, predicted and experimental values

Optimal condition			Galantamine content %	
Ultrasound time (min.)	**Ultrasound Temperature (°C)**	**Solvent/Material Ratio (mL/g Sample)**	**Experimental**	**Predicted**
32.89	51.04	40.70	0.470	0.469

4. CONCLUSİON

In this study, all the statistical indicators supported that RSM is a successful tool to describe the ultrasonic process in extracting galantamine from the bulbs of *G. woronowii L.* for the following tested ultrasonic parameters; ultrasound time (15-45 min) ultrasound temperature (30-70°C) and solvent/material ratio (30-50 mL/g sample) at a frequency of 50 kHz. The response dependent variable as represented by the yield of extracted galantamine could be expressed by a quadratic polynomial model and according to the analysis of variance and regression coefficients. The optimal theoretical extraction conditions were recorded as follows: extraction time, 32.89 min and temperature, 51.04°C, solvent/material ratio, 40.70 mL/g sample. Under the optimal conditions, the predicted optimal yield of extracted galantamine was 0.469%. By applying these conditions, the actual experimental value of extracted galantamine yield was 0.470% which is in agreement with the theoretically predicted one. These outcomes indicated the adequacy of the quadratic polynomial model to represent the ultrasonic extraction of galantamine for the variables within the ranges of the investigation. It has been evidenced that UAE combined with RSM is an effective technique for the extraction of natural galantamine from the bulbs of *G. woronowii L.* The results should be useful for full utilisation of *G. woronowii L.* and also indicated that UAE could be used as an efficient method for the extraction of natural galantamine from plant materials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETİNG İNTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Willis JC, Amaryllidaceae. In: Shaw AHK, editor. A dictionary of the flowering plants & ferns. 8th ed. Cambridge: Cambridge University Press; 1988.
2. Davis AP. The genus galanthus-snowdrops in the wild. In: Bishop M, Davis AP, Grimshaw J. Snowdrops, a Monograph of Cultivated Galanthus. Cheltenham: Griffin Press Publishing Ltd; 2006.
3. Yakovleva AP. Alkaloids of galanthus woronowii. J Gen Chem. 1963;33:1691-1693.
4. Kintsurashvili L, Vachnadze V. Plants of the amaryllidaceae family grown and introduced in Georgia: A source of galanthamine. Pharm Chem J-USSR. 2007;41:492-494.
5. Samuelsson G. Drugs of Natural Origin. A Textbook of Pharmacognosy. Stockholm: Swedish Pharmaceutical Press; 1992.
6. Cronnin JR. The plant alkaloid galanthamine. Approved as a drug; Sold as asupplement. Altern Complement Therapy. 2001;7(6):380–383.
7. Bulavka V, Tolkachev O. Synthesis of galanthamine and related compounds, in: Hanks GR, editör. Narcissus and Daffodil, New York: Taylor & Francis; 2002.
8. Hille T, Hoffmann HR, Kreh M, Matusch R. Process for the isolation of galanthamine, Patent No: 6,573,376 B2, Lts Lohmann Therapie-Systeme Ag; 2002.
9. Chemat F, Khan MK. Applications of ultrasound in food technology: Processing, preservation and extraction. Ultrason Sonochem. 2011;18:813–835.
10. Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. Int J Mol Sci. 2012;13(7):8615–8627.
11. Patis A, Bates D. Ultrasonic innovations in the food industry: From the laboratory to commercial production. Innov Food Sci Emerg Technol. 2008;9(2):147–154.
12. Hromadkova Z, Ebringerova A, Valachovic P. Comparison of classical and ultrasound-assisted extraction of polysaccharides from Salvia officinalis L. Ultrason Sonochem. 1999;5:163–168.
13. Shirsath S, Sonawane S, Gogate P. Intensification of extraction of natural products using ultrasonic irradiations – a review of current status. Chem Eng Process. 2012;53:10–23.
14. Zhao Q, Kennedy JF, Wang X, Yuan X, Zhao B, Peng Y. Optimization of ultrasonic circulating extraction of polysaccharides from asparagus officinalis using response surface methodology. Int J Biol Macromol. 2011;49:181–187.

15. Box GE, Behnken DW. Some new three level designs for the study of quantitative variables. Technometrics. 1960;2:455–475.
16. Ferreira SC, Bruns R, Ferreira H, Matos G, David J, Brandao G. Box-Behnken design: An alternative for the optimization of analytical methods. Anal Chim Acta. 2007;597:179–186.
17. Celli GB, Ghanem A, Brooks MSL. Optimization of ultrasound-assisted extraction of anthocyanins from haskap berries (*Lonicera caerulea L.*) using Response Surface Methodology. Ultrason Sonochem. 2015;27:449–455.
18. Wang W, Ma X, Xu Y, Cao Y, Jiang Z, Ding T et. al., Ultrasound-assisted heating extraction of pectin from grapefruit peel: Optimization and comparison with the conventional method. Food chem. 2015; 178:106–114.
19. Hemwimol S, Pavasant P, Shotipruk A. Ultrasound-assisted extraction of anthraquinones from roots of Morinda citrifolia. Ultrason Sonochem. 2006;13: 543–348.
20. Chen F, Zhang Q, Fei S, Gu H, Yang L. Optimization of ultrasonic circulating extraction of samara oil from Acer saccharum using combination of Plackett-Burman design and Box-Behnken design. Ultrason Sonochem. 2017;35:161–175.

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