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Research Article

EXTRACTION OPTIMIZATION OF *Senecio vernalis* Waldst. & Kit AND DETERMINATION OF ANTI-α-AMYLASE/α-GLUCOSIDASE, ANTI-LIPASE AND ANTIOXIDANT ACTIVITIES

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Abstract: The possible side effects of drugs used in type II diabetes are increasing the tendency to herbal resources that have been used for many years. *Senecio vernalis* Waldst. & Kit is one of the annual *Senecio* L. species widely distributed in Turkey and used as a food and folk medicine. In this study, optimization of extraction conditions on the bioactive properties (Total phenolic content (TPC) and antioxidant capacity) of the flowers of *S. vernalis* and the potential of the plant for α-amylase, α-glucosidase, and lipase inhibitory activity were investigated. The optimum extraction conditions were determined at 69.72% water concentration, 59°C for 26.15 min, and the highest experimental values of TPC and 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging activity were observed as 28.14 mg gallic acid equivalent (GAE) g^{-1} and 3165.99 mg trolox equivalent (TE)/100 g sample, respectively. Significant inhibition was observed for α-amylase and α-glucosidase which are the key enzymes in type II diabetes, at a concentration of 100 mg mL^{-1}, with 21.32% and 64.16% respectively. The *S. vernalis* extracts showed no detectable inhibition of lipase. The results showed that *S. vernalis*, which has high antioxidant capacity also has a significant anti-diabetic effect. It can be concluded that *S. vernalis* can be considered a natural resource in many industries such as food and pharmaceuticals.

Özet: Tip II diyabette kullanılan ilaçların olası yan etkileri, uzun yıllardır kullanılan bitkisel kaynaklara olan eğilimi arttırmaktadır. *Senecio vernalis* Waldst. & Kit, Türkiye'de yaygın olarak bulunan, gıda ve halk ilacı olarak kullanılan tek yıllık *Senecio* L. türlerinden biridir. Bu nedenle, bu çalışmada, *S. vernalis* çiçeklerinin biyoaktif özellikleri (Toplam fenolik madde miktarı (TPC) ve antioksidan kapasite) ve α-amilaz, α-glukozidaz ve lipaz inhibitör aktivite potansiyeli üzerinde optimizasyon ekstraksiyon koşulları araştırıldı. Optimum ekstraksiyon koşulları %69.72 su konsantrasyonunda, 59°C'de 26.15 dakika olarak belirlenmiş ve TPC ve 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) süpürme aktivitesinin en yüksek deneysel değerleri sırasıyla 28,14 mg gallik asit eşdeğeri (GAE) g^{-1} ve 3165.99 mg troloks eşdeğeri (TE)/100 g numune olarak belirlenmiştir. Tip II diyabette anahtar enzim olan α-amilaz, α-glukozidaz için 100 mg mL^{-1} konsantrasyonunda sırasıyla %21.32 ve %64.16 inhibisyon gözlemlendi. *Senecio vernaris* ekstraktı, saptanabilir bir lipaz inhibisyonu göstermedi. Sonuçlar, yüksek bir antioksidan kapasiteye sahip olan *S. vernalis*'in de önemli bir anti-diyabetik etkiye sahip olduğunu göstermiştir. *Senecio vernalis*'in gıda ve ilaç gibi birçok endüstride doğal bir kaynak olarak değerlendirilebileceği sonucuna varılabilir.

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Introduction

Throughout human history, many diseases have been tried to be treated using herbal cures. Scientific evidence supporting the effects of traditionally used herbs due to their beneficial features has brought these plants into the center of attention again. The World Health Organization (WHO) reports that approximately 80% of the world's population tries to overcome their health problems with herbal resources as the leading treatment agent (Anonymous 2000). Besides, active ingredients of plant origin constitute approximately 25% of prescription drugs in developed countries (Mosihuzzaman & Choudhary 2008). Various plant extracts have vast usage potential in various sectors such as nutraceuticals, pharmaceuticals, food additives and natural pesticides (Anklam *et al.* 1998). The health benefits of plants are mostly related to bioactive compounds, which are their secondary metabolites (Bernhoft 2010). A large number of studies were performed on the rich bioactive components

contained in plants (Azmir *et al.* 2013, Pereira *et al.* 2017). Extraction parameters are vital to benefit from the bioactive components possessed by plants at the highest level (Sasidharan *et al.* 2011). It is crucial to optimize extraction factors such as the solvent type, temperature, and time for the extraction to be effective (Başyiğit *et al.* 2020). Response surface methodology (RSM) successfully combines mathematical and statistical techniques applied with a minimum trial point in optimizing extraction factors (Myers *et al.* 2016).

Diabetes mellitus (DM) is a disease that affects 285 million people worldwide in 2010 and is predicted to affect more than 400 million people by the year 2030. Type II diabetes, which is mainly affected by environmental factors such as diet and lifestyle, has a very high effect on the increase in reported cases (Wild *et al.* 2004). Type II diabetes is a metabolic disorder that affects 90% of diabetes patients and causes an uncontrolled increase in blood sugar (Bhutkar & Bhise 2012). Although this increase in blood glucose level can be regulated by therapeutic drugs, the treatment solution of conscious patients with herbal supplements appears to be an up-to-date approach considering the possible side effects of medical drugs (Cariou *et al.* 2012). During the last couple of decades, *in vitro* and *in vivo* studies on alpha-amylase and alpha-glucosidase inhibition with various food, food components and herbal supplements to reduce glucose absorption have been performed (Matsui *et al.* 1996, Lee *et al.* 2007, Doğan *et al.* 2021).

Senecio L. is a large and diverse genus in the *Asteraceae* family with approximately 1500 described species widely known all over the world (Christov *et al.* 2002). The genus is represented with 39 species in Turkey (Uğur *et al.* 2006). These species are generally called as "Canary grass" and rarely as "Küllüce grass" and "Ekin grass" in Turkey (Baytop 2007). *Senecio* species have long been consumed as food or folk remedies with their antiemetic, anti-inflammatory, and vasodilator properties (Conforti *et al.* 2006a). In addition, some species are known with their antibacterial-antifungal (Kiprono *et al.* 2000), antimicrobial-cytotoxic (Loizzo *et al.* 2006), antioxidant and anti-diabetic activities (Ayoola *et al.* 2019).

The present study was performed to determine the effects of optimized extraction conditions on total phenolic content and antioxidant capacity of *S. vernalis* flowers. Anti-diabetic and anti-lipase activity, which have not been evaluated in previous studies, were also evaluated.

Materials and Methods

Plant material and treatments

Senecio vernalis was collected from Yozgat Bozok University Boğazlıyan Vocational School campus in Turkey (N39°20'25.62", E35°26'07.84"). The collected samples were separated from their flowers and dried at 40°C until they reached constant weight. Before the extraction, flower samples were ground through a laboratory steel blender (Waring 8011, USA) for 1 min. The chemicals used in the analysis were obtained from Merck (Darmstadt, Germany) unless otherwise indicated. α-amylase, α-glucosidase, and lipase were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Creating the experimental design and extraction

The effects of temperature (40-60°C), time (5-60 min) and solvent concentration (water to ethanol: 0-100%) as the extraction conditions (independent variables) on Total phenolic content (TPC) and 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) were determined by Design Expert 11.0.0 software (Stat- Ease Inc., Minneapolis, MN) using a face-centered central composite design (FC-CCD). The effects of the extraction conditions on the responses (TPC and DPPH) were expressed by the following quadratic polynomial regression equation (Eq. 1):

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} {X_1}^2 + \beta_{22} {X_2}^2 + \beta_{33} {X_3}^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \ (Eq. 1)$$

where Y is the predicted response (TPC and DPPH), β_0 is the constant, β_1, β_2, β_3 are the linear coefficients, β_{11}, β_{22}, β_{33} are the interaction coefficients, β_{12}, β_{13}, β_{23} are quadratic coefficients, and X_1 (temperature), X_2 (time) and X_3 (solvent concentration) are the independent variables. The whole design was created at 20 experimental points, and the level of independent variables, experimental values, and estimated data was given in Table 1.

For extraction, 0.5 g of sample was mixed with 10 ml of solvent and extracted according to the experimental point. The extracted samples were centrifuged at 5000 rpm for 5 min and the supernatant was collected and stored at -18°C.

Total phenolic content (TPC) assay

0.4 mL sample was mixed with diluted 2 mL Folin-Ciocalteu reagent and 1.6 mL Na_2CO_3 (7.5%) in a test tube. After the mixture was incubated in dark for 60 min, the absorbance was read in the spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) at 765 nm. The absorbance values obtained are expressed in gallic acid equivalent (GAE) (Singleton *et al.* 1999).

Antioxidant activity assay

DPPH method was used to determine the antioxidant capacity of the samples. For this purpose, a 0.1 g sample was mixed with 3.9 mL of DPPH solution (25 mg/L) prepared with methanol in a test tube. After 30 min of incubation in dark, absorbances at 515 nm were recorded using a Shimadzu UV-1700 spectrophotometer (Shimadzu UV-1700, Kyoto, Japan) (Brand-Williams *et al.* 1995). Results are expressed as trolox equivalent (mg TE/100 g sample).

In vitro anti-diabetic activity assays

The anti-diabetic activity of the samples was determined considering the α-amylase and α-glucosidase inhibitory activity. For the α-amylase inhibition test of the samples, after keeping 1 mL of an extract with 1 mL of potato starch and $NaHPO_4$ (20 mM) at 37°C for 5 min, the

reaction was started by adding 1 mL α-amylase. After 30 min of incubation, 0.5 mL of Rochella Salt (5.31 M) and 0.5 mL of 3,5-dinitrosalicylic acid (96 mM) solution were added. The mixture was terminated by standing at 100°C for 15 min. After heat treatment, the absorbance of the mixture was recorded at 540 nm. For the α-glucosidase inhibition test, after mixing 50 µL extract and 1250 µL 67 mM KH_2PO_4 with 50 µL α-glucosidase in a test tube, it was incubated at 37°C for 5 min. Afterward, 125 µL of p-Nitrophenyl-β-D-glucopyranoside (10 mM) solution was added, and the reaction was started and terminated by adding 2 mL of Na_2CO_3 (100 mM) solution after 20 min. The absorbance of the mixture was recorded at 400 nm (McDougall *et al.* 2005a, Cam *et al.* 2020). Absorbances were recorded using the spectrophotometer (Shimadzu UV 1700, Tokyo, Japan) to determine the inhibitory activity of both enzymes.

The α-amylase and α-glucosidase inhibitory activities were expressed as a percentage of inhibition and the following formula was used to determine enzyme inhibitory activity (%) of the samples (Eq. 2).

$$Enzyme\ inhibition\ (\%) = \frac{ABS_{control} - ABS_{sample}}{ABS_{control}} x100\ (Eq.2)$$

where $ABS_{control}$ and ABS_{sample} express the absorbance of the control and samples, respectively.

Lipase inhibition activity

The lipase inhibition activity of the diluted samples was evaluated *in vitro* using the spectrophotometric method. This assay was performed using the method by Gilham & Lehner (2005). Porcine pancreas lipase (10 mg/mL) was prepared as the enzyme solution. 800 µL of 100 mM Tris buffer (pH = 8.2) was mixed in a test tube with 100 µL of diluted extract and 300 µL of the prepared lipase solution. After incubation for 5 min at 37°C, 800 µL of p-nitrophenyl-laurate (300 µg/mL) was added. p-nitrophenyl-laurate is a colored compound and absorbs at 400 nm and through this compound, the enzyme activity is read in the spectrophotometer (Shimadzu UV 1700). The control and blank samples were prepared in the same way by subtracting the extract and both of the extract and enzyme, respectively (Eq. 3).

$$Lipase\ inhibition\ activity\ (\%) = \frac{Abs_{control} - Abs_{extract}}{Abs_{control}} x100\ (Eq.3)$$

where $Abs_{control}$ and $Abs_{extract}$ express the absorbances of the control and extract, respectively.

Statistical analysis

To determine the reliability of the 2nd-order polynomial equations derived from the model, Regression (p-value), coefficient of determination (R^2), adjusted R^2 (R^2_{adj}), predicted R^2 (R^2_{pred}), and lack of fit were demonstrated using Design Expert 11.0.0 software (Stat-Ease Inc., Minneapolis, MN). SPSS 22.0 software (SPSS Inc., Chicago, IL) was used for all data analyses where $p<0.05$ was assumed to be statistically significant. Principle component analysis (PCA) used to determine the correlation between data was performed with Minitab 18 software (Minitab Inc., PA, USA).

Result and Discussion

Checking the model fitting

The experimental value and the predicted data performed at the experimental points created according to the FC-CCD result were given in Table 1.

Table 1. Experimental values and the predicted data according to FC-CCD.

Experimental point	Independent variables			Responses			
	X_1 (°C)	X_2 (min)	X_3 (%)	TPC (mg GAE g^{-1})		DPPH (mg TE/100 g sample)	
				Experimental value	Predicted data	Experimental value	Predicted data
1	40	5	0	2.68	2.70	163.60	166.18
2	50	32.5	50	25.80	25.52	2880.78	2998.21
3	60	60	0	1.86	1.96	114.56	117.13
4	50	32.5	50	25.62	25.52	2949.75	2998.21
5	40	60	100	20.59	20.14	2034.22	1990.33
6	60	5	100	18.19	18.70	1385.79	1404.21
7	60	5	0	2.09	2.02	136.70	134.82
8	60	60	100	19.10	18.09	1553.35	1614.77
9	50	32.5	50	24.24	25.52	2997.25	2998.21
10	40	5	100	24.98	24.93	1783.16	1730.80
11	40	60	0	2.12	2.18	142.49	144.37
12	50	32.5	50	25.05	25.52	2915.26	2998.21
13	50	32.5	0	3.00	2.85	191.42	185.14
14	50	32.5	50	26.28	25.52	2907.98	2998.21
15	60	32.5	50	18.82	19.04	2333.28	2204.81
16	50	60	50	22.36	22.42	2904.52	2766.77
17	40	32.5	50	23.36	23.2	2646.29	2717.60
18	50	32.5	100	24.98	26.38	2211.08	2218.46
19	50	32.5	50	26.22	25.22	3144.74	2998.21
20	50	5	50	25.31	25.36	2717.17	2768.04

Table 2. 2nd-order polynomial equations and statistical parameters for model fitness.

Responses	2nd-order polynomial equations	Regression (p-value)	R^2	R^2_{adj}	R^2_{pred}
TPC	=-3.067+0.178*temperature-0.0045*time+0.065*solvent concentration +0.00016*temperaturetime-0.0019*temperature^2-0.000089*time^2-0.00043*solvent concentration^2	<0.0001	0.999	0.998	0.997
DPPH	=0.643+0.192*temperature+0.0043*time+0.084*solvent concentration +5.09735e-05*time solvent concentration-0.002*temperature^2-0.0001*time^2-0.0006*solvent concentration^2	<0.0001	0.999	0.999	0.998

Table 3. Analysis of variance for responses.

DPPH					TPC				
Source	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	**Source**	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**
Model	30.43	4.35	2940.88	< 0.0001	**Model**	20.32	2.90	1839.99	< 0.0001
X_1 Temperature	0.1093	0.1093	73.96	< 0.0001	X_1 Temperature	0.0977	0.0977	61.89	< 0.0001
X_2 Time	5.260E-07	5.260E-07	0.0004	0.9853	X_2 Time	0.0381	0.0381	24.13	0.0004
X_3 Solvent concentration	15.42	15.42	10432.69	< 0.0001	X_3 Solvent concentration	12.37	12.37	7836.90	< 0.0001
X_2X_3	0.0393	0.0393	26.59	0.0002	X_1X_2	0.0162	0.0162	10.28	0.0076
X_1^2	0.1131	0.1131	76.54	< 0.0001	X_1^2	0.1036	0.1036	65.69	< 0.0001
X_2^2	0.0176	0.0176	11.94	0.0048	X_2^2	0.0127	0.0127	8.02	0.0151
X_3^2	6.55	6.55	4429.63	< 0.0001	X_3^2	3.20	3.20	2028.31	< 0.0001
Residual	0.0177	0.0015			**Residual**	0.0189	0.0016		
Lack of Fit	0.0126	0.0018	1.77	0.2743	Lack of Fit	0.0142	0.0020	2.16	0.2063
Pure Error	0.0051	0.0010			Pure Error	0.0047	0.0009		
Cor Total	30.44				**Cor Total**	20.34			

The 2nd -order polynomial equations derived from the model and its statistical parameters were given in Table 2. To ensure the reliability of the model, firstly insignificant terms were removed from the polynomial equation. For this purpose, the automatic model selection module of the Design Expert software is used to algorithmically select the terms to be kept in the model. To determine whether there is an unimportant term in the model, the Adjusted R-square selection, which follows one step backwards at a time and removes the least significant term from the model was preferred. This is very important in determining the impact of important factors on responses (Hastie *et al.* 2001). In addition, it is recommended that the difference between R^2_{adj} and R^2_{pred} to be less than 0.2 and R^2 and R^2_{adj} values above 90% in determining the suitability of the model (Myers *et al.* 2004). In addition, the model should not have a lack of fit. P-value of the lack of fit for the TPC and DPPH of the samples was determined as 0.206 and 0.274, respectively, in other words no model lack of fit was detected (Table 3). Additionally, as shown in Table 2, R^2, R^2_{adj}, and R^2_{pred} values are greater than 90%, and the differences between R^2_{adj} and R^2_{pred} values are less than 0.2.

Effects of the extraction conditions on TPC and antioxidant activity

The results of TPC and DPPH are presented in Table 1. When the effects of extraction conditions on TPC and DPPH are examined, while temperature and solvent concentration were significant for both ($p<0.05$), time was significant for TPC ($p<0.05$) but not for DPPH ($p>0.05$). The highest TPC and DPPH values in the extraction at 20 experimental points were detected with 26.28 GAE g-1 and 3144.74 mg TE/100 g sample at the midpoint (50°C, 32.50 min, and 50% ethanol), respectively. The lowest values were obtained with 1.86 GAE g-1 and 114.56 mg

TE/100 g sample in 100% ethanol solvent extraction at the experimental point where the temperature and time values were at maximum. One of the main objective of extraction should be to reduce the use of organic solvents as much as possible. For this purpose, binary solvent mixtures (water-ethanol) were tried rather than single-use of ethanol to extract secondary metabolites, and higher efficiency was obtained in its use. In addition, in studies evaluating the extraction performance, mixed solvents came to the fore (Markom *et al.* 2007). The amount of phenolic compounds in the extract increased up to 50°C but decreased rapidly in parallel with the increase in temperature (Fig. 1). In classical extraction, it is vital to increase the solubility of the tissues by softening the temperature. However, it is a known fact that high temperatures damage phenolics (Dent *et al.* 2013). The increase in time is thought to be insignificant for DPPH since the antioxidants in phenolic compounds pass into the extract until the 32.50th min and are not affected by the increase in time as much as phenolics after that min. By shortening the extraction time, energy wastage is prevented and time is saved in the process (Chew *et al.* 2011). Since the extraction efficiency will vary according to the phenolic compounds of the raw material, the extraction method and conditions, it is crucial to optimize it. In previous studies, some studies determined the TPC and antioxidant capacity of different *Senecio* species (Lone *et al.* 2014, Sharma & Shah 2015, Faraone *et al.* 2018, Ayoola *et al.* 2019). However, studies showing the bioactive properties of *S. vernalis* are extremely limited (Balpinar & Okmen 2019). In addition, the flowers of *S. vernalis* contain high amounts of carotenoids (Mogoşanu *et al.* 2009). The fact that carotenoids have reactive double bonds in conjugated structure gives them antioxidant properties (Suparmi & Prasetya 2012).

Principle component analysis (PCA)

To improve the interpretability of multivariate modelsPCA is a method that has been used frequently in recent years. PCA is a method of finding the projection of data in a multidimensional space onto a lower-dimensional space in a way that maximizes the variance (Alpaydin 2020). The HJ-biplot was constructed with the first (97.6%) and second (2.4%) components, contributing to all of the total variability. On the biplot, the correlation between the variables was expressed by the acute angle at the intersection of the vectors. Moreover, the relationship between 20 experimental points and variables was reflected with the HJ-biplot. Accordingly, the experimental points were divided into 3 groups expressed as a circle, triangle and square. The basis of the grouping was the solvent concentration. The 1st group (circle-shaped) with the lowest phenolic compound and antioxidant capacity was localized farthest away in the absence of water as a solvent. The second group (square-shaped) represents the experimental points where the solvent is 100% water, and since the TPC and DPPH values at these points are higher than the first group, they are closer to the intersection of the vectors. The third group (triangle-shaped), on the other hand, constitutes the large group that includes the midpoints of the test points, as well as the points taken with half the water-ethanol mixture as solvent, and the TPC and DPPH values obtained at these points are the highest. The findings show that the phenolics of the sample are better soluble in the binary solvent system and the aqueous extract is higher than ethanol in the use of a single solvent.

There is an intense relationship between the phenolic content of the plant materials and their antioxidant activities (Aryal *et al.* 2019). As can be seen from PCA, a positive correlation was determined between the phenolic compounds of *S. vernalis* and its antioxidants (Fig. 2).

Optimization and model validation

Optimum extraction conditions and both experimental values and predicted data at this point are presented in Table 3. Optimum extraction conditions were determined as 69.72% water concentration at 57.29°C for 26.15 min. The predicted data according to the model at the optimum point were observed as 28.14 mg GAE g^{-1} and 3165.99 mg TE/100 g sample for TPC and DPPH, respectively. In addition, the experimental values made at this point were determined as 27.94 mg GAE g^{-1} and 3054.77 mg TE/100 g samples for TPC and DPPH, respectively. As it is clear from the results, the predicted data and experimental values are in good agreement. Briefly, there is no statistically significant difference ($p>0.05$).

Anti-diabetic activity and lipase inhibition activity

Dilutions at 1, 2, 5, 10, 20, 50, 75, and 100 mg mL^{-1} were prepared from the extracts taken at the optimization point, and α-amylase, α-glucosidase, and lipase inhibition activities were evaluated. With increasing concentration from 1 to 100 mg mL^{-1}, the inhibition activities of α-amylase and α-glucosidase increased. The results showed that the inhibition activity of α-amylase and α-glucosidase ranged between 4.12%-21.32% and 17.94%-64.16% respectively. α-glucosidase inhibition activity was found to be higher than α-amylase inhibition activity. The reason for this situation is thought to be the bioactive compounds of *S. vernalis*. Wang *et al.* (2010) reported that seven pure flavonoid compounds showed an inhibitory effect on different enzymes. Pancreatic α-glucosidase and α-amylase are needed to convert complex carbohydrates to simple sugars in the gastrointestinal system. Inhibition of these enzymes is one of the methods applied for plasma glucose levels decreased in the blood (Krentz & Bailey 2005). The methods of inhibition of these enzymes and/or restriction of absorption of monosaccharides are utilized in currently used medicinal drugs such as acarbose, miglitol, voglibose, etc. (Dash *et al.* 2018). However, due to the known side effects of these drugs (Su *et al.* 2013), interest in natural agents with strong inhibitory effects and less side effects and/or no side effects has increased in recent years (Kim *et al.* 2004, Ali *et al.* 2006, Bhandari *et al.* 2008, Hung *et al.* 2012).

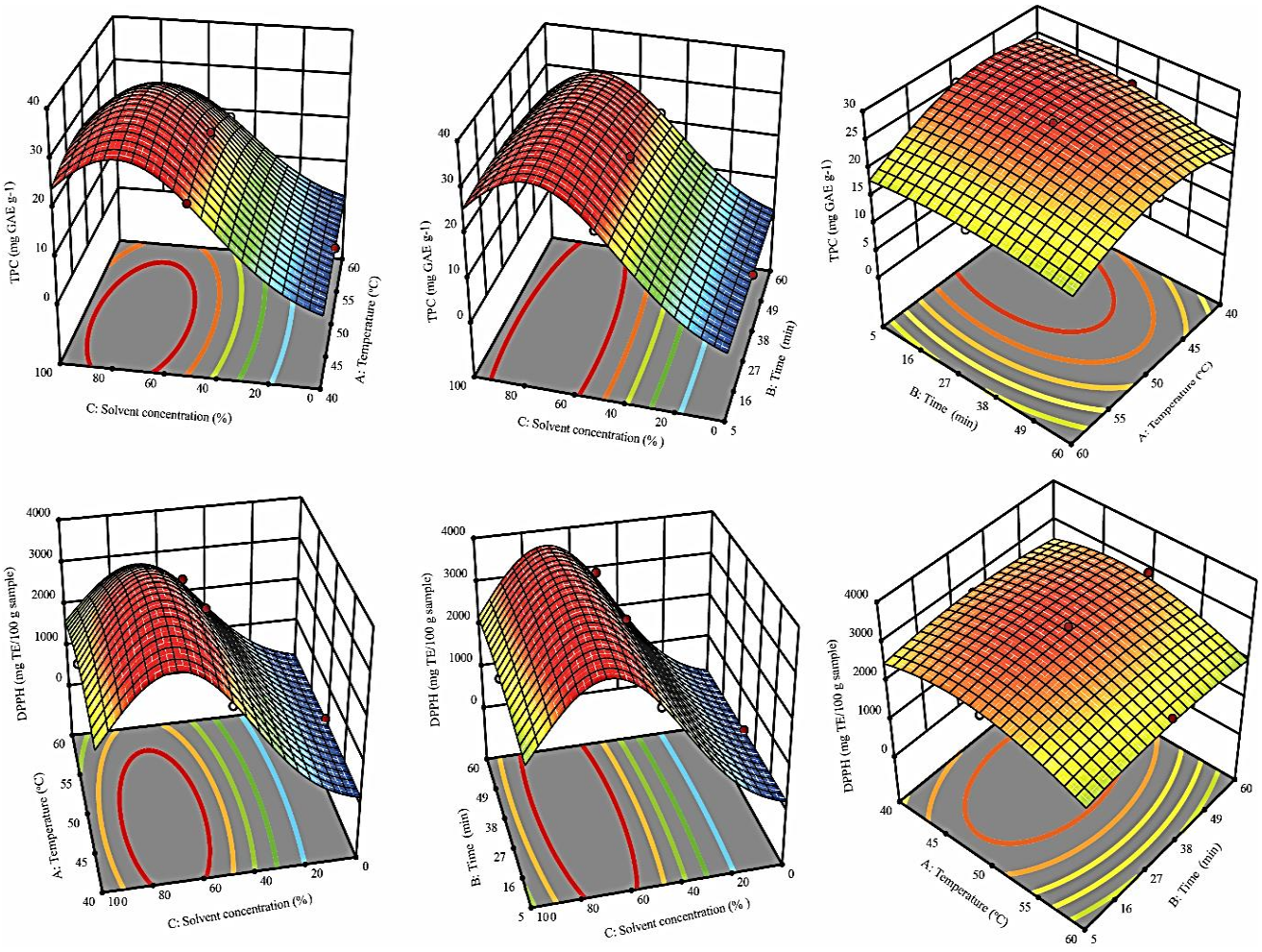

Fig. 1. Representation of the interaction effect of extraction conditions on responses with 3D surface plot.

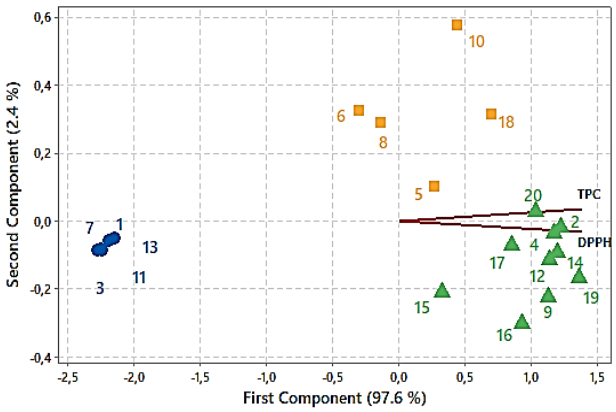

Fig 2. HJ-biplot of the distribution of experimental points over the responses for PCA.

The anti-diabetic activities of different *Senecio* species were determined in previous studies (Conforti *et al.* 2006b, Tundis *et al.* 2012, Ajiboye *et al.* 2018, Ma *et al.* 2018b). However, no study was found to determine the α-amylase and α-glucosidase inhibition capacity of *S. vernalis*. Hyperglycemia is highly correlated with oxidative stress and the activity of key enzymes such as pancreatic α-amylase α-glucosidase (Hung *et al.* 2017). In previous *in vivo* and *in vitro* studies, it was emphasized that oxidative stress causes dysfunction in β-cells responsible for glucose metabolism (Robertson 2004, Tang *et al.* 2012, Chang *et al.* 2013). Therefore, oxidative stress should be reduced as much as possible to prevent or reduce diabetic complications (DeFronzo 1999). Antioxidants play an essential role in avoiding related disorders such as degenerative diseases, diabetes and cancer by controlling oxidative stress (Birben *et al.* 2012). Some studies suggest that the progression of type 2 diabetes can be reduced by consuming diets rich in plant-based antioxidants (Faller & Fialho 2009, Porter 2012). In addition, diabetes is one of the oxidative stress states in which free radicals increase and antioxidant mechanisms are inhibited. Therefore, it is recommended to use anti-diabetics with antioxidant properties to treat diabetes (Memişoğulları 2005). It is also known that plant polyphenols and antioxidants have effective anti-diabetic properties (McDougall *et al.* 2005b, Mai *et al.* 2007). Therefore, in this study, *S. vernalis* was extracted at the point where its antioxidant capacity was at its maximum. Then the inhibition capacities of α-amylase α-glucosidase were investigated. Consequently, it is thought that the high antioxidant activity of *S. vernalis* and its anti-diabetic effect provide dual benefits. None of the extracts showed dose-dependent inhibition of lipase enzymes.

Table 4. Optimum extraction conditions with experimental values and predicted data at these conditions.

Temperature (°C)	Time (min)	Solvent concentration (%)	Desirability score	Responses	Predicted data	Experimental value
57.29	26.15	69.72	1.00	TPC (mg GAE g^{-1})	28.14	27.94
				DPPH (mg TE/100 g sample)	3165.99	3054.77

Conlusion

RSM has been successfully applied to optimise extraction on TPC and antioxidant activity of *S. vernalis* flowers. The most effective extraction conditions were determined as 69.72% water concentration, 59°C for 26.15 min with which the experimental values of TPC and DPPH were observed as 28.14 mg GAE g^{-1} and 3165.99 mg TE/100 g sample, respectively. Extracts at various concentrations exhibited not only antioxidant but also potential α-glucosidase and α-amylase inhibitory activity. Therefore, this extract may be promising for a therapeutic approach in the management of type II diabetes, as it has anti-diabetic potential as well as high antioxidant activity.

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References

1. Anonymous. 2000. General *Guidelines for Methodologies on Research and Evaluation of Traditional Medicine*, World Health Organization, Geneva, 79 pp.
2. Ajiboye, B.O., Ojo, O.A., Okesola, M.A., Akinyemi, A.J., Talabi, J.Y., Idowu, O.T., Fadaka, A.O., Boligon, A.A. & Anraku de Campos, M.M. 2018. In vitro antioxidant activities and inhibitory effects of phenolic extract of *Senecio biafrae* (Oliv and Hiern) against key enzymes linked with type II diabetes mellitus and Alzheimer's disease. *Food Science & Nutrition*, 6(7): 1803-1810.
3. Ali, H., Houghton, P. & Soumyanath, A. 2006. α-Amylase inhibitory activity of some Malaysian plants used to treat diabetes; with particular reference to *Phyllanthus amarus*. *Journal of Ethnopharmacology*, 107(3): 449-455.
4. Alpaydin, E. 2020. *Introduction to Machine Learning*. MIT Press, Massachusetts, 712 pp.
5. Anklam, E., Berg, H., Mathiasson, L., Sharman, M. & Ulberth, F. 1998. Supercritical fluid extraction (SFE) in food analysis: a review. *Food Additives & Contaminants*, 15(6): 729-750.
6. Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R. & Koirala, N. 2019. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, 8(4): 96.
7. Ayoola, M., Adebajo, A., Zotor, F. & Pinkoane, M. 2019. Justifying antidiabetic ethnomedicinal claim of *Senecio biafrae* through its antihyperglycemic and anti-oxidant activities. *Annals of Complementary and Alternative Medicine*, 1(2): 1006.
8. Azmir, J., Zaidul, I.S.M., Rahman, M., Sharif, K., Mohamed, A., Sahena, F., Jahurul, M., Ghafoor, K., Norulaini, N. & Omar, A. 2013. Techniques for extraction of bioactive compounds from plant materials: A review. *Journal of Food Engineering*, 117(4): 426-436.
9. Balpinar, N. & Okmen, G. 2019. Biological activities and chemical composition of *Senecio vernalis* growing in the lakes region of Turkey. *International Journal of Environmental Science and Technology*, 16(9): 5205-5212.
10. Başyiğit, B., Alaşalvar, H., Doğan, N., Doğan, C., Berktaş, S. & Çam, M. 2020. Wild mustard (*Sinapis arvensis*) parts: compositional analysis, antioxidant capacity and determination of individual phenolic fractions by LC–ESI–MS/MS. *Journal of Food Measurement and Characterization*: 1-11.
11. Baytop, T. 2007. *Türkçe Bitkı Adları Sözlüğü*. Türk Dil Kurumu Yayınları, Ankara, 512 pp.
12. Bernhoft, A. 2010. A brief review on bioactive compounds in plants. *Bioactive Compounds in Plants-Benefits and Risks for Man and Animals*, 50: 11-17.
13. Bhandari, M.R., Jong-Anurakkun, N., Hong, G. & Kawabata, J. 2008. α-Glucosidase and α-amylase inhibitory activities of Nepalese medicinal herb Pakhanbhed (*Bergenia ciliata*, Haw.). *Food Chemistry*, 106(1): 247-252.
14. Bhutkar, M. & Bhise, S. 2012. In vitro assay of alpha amylase inhibitory activity of some indigenous plants. *International Journal of Chemical Sciences*, 10(1): 457-462.
15. Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S. & Kalayci, O. 2012. Oxidative stress and antioxidant defense. *World Allergy Organization Journal*, 5(1): 9-19.
16. Brand-Williams, W., Cuvelier, M.E. & Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1): 25-30.

17. Cam, M., Basyigit, B., Alasalvar, H., Yilmaztekin, M., Ahhmed, A., Sagdic, O., Konca, Y. & Telci, I. 2020. Bioactive properties of powdered peppermint and spearmint extracts: Inhibition of key enzymes linked to hypertension and type 2 diabetes. *Food Bioscience,* 35: 100577.

18. Cariou, B., Charbonnel, B. & Staels, B. 2012. Thiazolidinediones and PPARγ agonists: time for a reassessment. *Trends in Endocrinology & Metabolism,* 23(5): 205-215.

19. Chang, C.L., Lin, Y., Bartolome, A.P., Chen, Y.C., Chiu, S.C. & Yang, W.C. 2013. Herbal therapies for type 2 diabetes mellitus: chemistry, biology, and potential application of selected plants and compounds. *Evidence-Based Complementary and Alternative Medicine,* 2013: 388657.

20. Chew, K., Khoo, M., Ng, S., Thoo, Y.Y., Aida, W.W. & Ho, C.W. 2011. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of Orthosiphon stamineus extracts. *International Food Research Journal,* 18(4): 1427.

21. Christov, V., Mikhova, B., Alexandrova, R., Dimitrova, D., Nikolova, E. & Evstatieva, L. 2002. Alkaloids from the roots of *Senecio macedonicus* Griseb. *Zeitschrift für Naturforschung C,* 57(9-10): 780-784.

22. Conforti, F., Loizzo, M.R., Statti, G.A., Houghton, P.J. & Menichini, F. 2006a. Biological properties of different extracts of two *Senecio* species. *International Journal of Food Sciences and Nutrition,* 57(1-2): 1-8.

23. Conforti, F., Marrelli, M., Statti, G. & Menichini, F. 2006b. Antioxidant and cytotoxic activities of methanolic extract and fractions from *Senecio gibbosus* subsp. gibbosus (GUSS) DC. *Natural Product Research,* 20(9): 805-812.

24. Dash, R.P., Babu, R.J. & Srinivas, N.R. 2018. Reappraisal and perspectives of clinical drug–drug interaction potential of α-glucosidase inhibitors such as acarbose, voglibose and miglitol in the treatment of type 2 diabetes mellitus. *Xenobiotica,* 48(1): 89-108.

25. DeFronzo, R.A. 1999. Pharmacologic therapy for type 2 diabetes mellitus. *Annals of Internal Medicine,* 131(4): 281-303.

26. Dent, M., Dragović-Uzelac, V., Penić, M., Bosiljkov, T. & Levaj, B. 2013. The effect of extraction solvents, temperature and time on the composition and mass fraction of polyphenols in Dalmatian wild sage (*Salvia officinalis* L.) extracts. *Food Technology and Biotechnology,* 51(1): 84-91.

27. Doğan, N., Doğan, C. & Atila, F. 2021. Parts from life-cycle of *H. erinaceus*: response surface methodology approach to optimize extraction conditions and determination of its antioxidant, antidiabetic and antimicrobial effect. *Journal of Microbiology, Biotechnology and Food Sciences*, e3703.

28. Faller, A. & Fialho, E. 2009. The antioxidant capacity and polyphenol content of organic and conventional retail vegetables after domestic cooking. *Food Research International,* 42(1): 210-215.

29. Faraone, I., Rai, D.K., Chiummiento, L., Fernandez, E., Choudhary, A., Prinzo, F. & Milella, L. 2018. Antioxidant Activity and Phytochemical Characterization of *Senecio* clivicolus Wedd. *Molecules,* 23(10): 2497.

30. Gilham, D. & Lehner, R. 2005. Techniques to measure lipase and esterase activity in vitro. *Methods,* 36(2): 139-147.

31. Hastie, T., Tibshirani, R. & Friedman, J. 2001. The elements of statistical learning. *Springer series in statistics*, Springer, New York, 764 pp.

32. Hung, H.Y., Qian, K., Morris-Natschke, S.L., Hsu, C.S. & Lee, K.H. 2012. Recent discovery of plant-derived anti-diabetic natural products. *Natural Product Reports,* 29(5): 580-606.

33. Hung, W.C., Ling, X.H., Chang, C.C., Hsu, H.F., Wang, S.W., Lee, Y.C., Luo, C., Lee, Y.T. & Houng, J.Y. 2017. Inhibitory effects of Siegesbeckia orientalis extracts on advanced glycation end product formation and key enzymes related to metabolic syndrome. *Molecules,* 22(10): 1785.

34. Kim, Y.M., Wang, M.H. & Rhee, H.I. 2004. A novel α-glucosidase inhibitor from pine bark. *Carbohydrate Research,* 339(3): 715-717.

35. Kiprono, P.C., Kaberia, F., Keriko, J.M. & Karanja, J.N. 2000. The in vitro anti-fungal and anti-bacterial activities of β-sitosterol from *Senecio lyratus* (Asteraceae). *Zeitschrift für Naturforschung C,* 55(5-6): 485-488.

36. Krentz, A.J. & Bailey, C.J. 2005. Oral antidiabetic agents. *Drugs,* 65(3): 385-411.

37. Lee, S.K., Hwang, J.Y., Song, J.H., Jo, J.R., Kim, M.J., Kim, M.E. & Kim, J.I. 2007. Inhibitory activity of Euonymus alatus against alpha-glucosidase in vitro and in vivo. *Nutrition Research and Practice,* 1(3): 184.

38. Loizzo, M., Tundis, R., Statti, G., Miljkovic-Brake, A., Menichini, F. & Houghton, P. 2006. Bioactive extracts from *Senecio samnitum* Huet. *Natural Product Research,* 20(3): 265-269.

39. Lone, S.H., Bhat, K.A., Bhat, H.M., Majeed, R., Anand, R., Hamid, A. & Khuroo, M.A. 2014. Essential oil composition of *Senecio graciliflorus* DC: Comparative analysis of different parts and evaluation of antioxidant and cytotoxic activities. *Phytomedicine,* 21(6): 919-925.

40. Ma, L., Lin, Q., Lei, D., Liu, S., Wang, X. & Zhao, Y. 2018. Alpha-glucosidase inhibitory activities of essential oils extracted from three chinese herbal medicines. *Chemical Engineering Transactions,* 64: 61-66.

41. Mai, T.T., Thu, N.N., Tien, P.G. & Van Chuyen, N. 2007. Alpha-glucosidase inhibitory and antioxidant activities of Vietnamese edible plants and their relationships with polyphenol contents. *Journal of Nutritional Science and Vitaminology,* 53(3): 267-276.

42. Markom, M., Hasan, M., Daud, W.R.W., Singh, H. & Jahim, J.M. 2007. Extraction of hydrolysable tannins from Phyllanthus niruri Linn.: Effects of solvents and extraction methods. *Separation and Purification Technology,* 52(3): 487-496.

43. Matsui, T., Yoshimoto, C., Osajima, K., Oki, T. & Osajima, Y. 1996. In vitro survey of α-glucosidase inhibitory food

components. *Bioscience, Biotechnology, and Biochemistry,* 60(12): 2019-2022.

44. McDougall, G.J., Dobson, P., Smith, P., Blake, A. & Stewart, D. 2005a. Assessing potential bioavailability of raspberry anthocyanins using an in vitro digestion system. *Journal of Agricultural and Food Chemistry,* 53(15): 5896-5904.

45. McDougall, G.J., Shpiro, F., Dobson, P., Smith, P., Blake, A. & Stewart, D. 2005b. Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. *Journal of Agricultural and Food Chemistry,* 53(7): 2760-2766.

46. Memişoğulları, R. 2005. Diyabette serbest radikallerin rolü ve antioksidanların etkisi. *Düzce Tıp Fakültesi Dergisi,* 7(3): 30-39.

47. Mogoşanu, G., Pintea, A., Bejenaru, L.E., Bejenaru, C., Rau, G. & Popescu, H. 2009. HPLC analysis of carotenoids from *Senecio vernalis* and *S. jacobaea* (Asteraceae). *Farmacia,* 57(6): 780-786.

48. Mosihuzzaman, M. & Choudhary, M.I. 2008. Protocols on safety, efficacy, standardization, and documentation of herbal medicine (IUPAC Technical Report). *Pure and Applied Chemistry,* 80(10): 2195-2230.

49. Myers, R.H., Montgomery, D.C. & Anderson-Cook, C.M. 2016. *Response Surface Methodology: process and product optimization using designed experiments*. John Wiley & Sons, New York, 704 pp.

50. Myers, R.H., Montgomery, D.C., Vining, G.G., Borror, C.M. & Kowalski, S.M. 2004. Response surface methodology: a retrospective and literature survey. *Journal of Quality Technology,* 36(1): 53-77.

51. Pereira, C.G., Barreira, L., da Rosa Neng, N., Nogueira, J.M.F., Marques, C., Santos, T.F., Varela, J. & Custódio, L. 2017. Searching for new sources of innovative products for the food industry within halophyte aromatic plants: In vitro antioxidant activity and phenolic and mineral contents of infusions and decoctions of *Crithmum maritimum* L. *Food and Chemical Toxicology,* 107(1): 581-589.

52. Porter, Y. 2012. Antioxidant properties of green broccoli and purple-sprouting broccoli under different cooking conditions. *Bioscience Horizons: The International Journal of Student Research,* 5:hzs004.

53. Robertson, R.P. 2004. Chronic oxidative stress as a central mechanism for glucose toxicity in pancreatic islet beta cells in diabetes. *Journal of Biological Chemistry,* 279(41): 42351-42354.

54. Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K. & Latha, L.Y. 2011. Extraction, isolation and characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complementary and Alternative Medicines,* 8(1): 1-10.

55. Sharma, P. & Shah, G. 2015. Composition and antioxidant activity of *Senecio nudicaulis* Wall. ex DC.(Asteraceae): a medicinal plant growing wild in Himachal Pradesh, India. *Natural Product Research,* 29(9): 883-886.

56. Singleton, V.L., Orthofer, R. & Lamuela-Raventós, R.M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology,* 299(1): 152-178.

57. Su, C.H., Lai, M.N. & Ng, L.T. 2013. Inhibitory effects of medicinal mushrooms on α-amylase and α-glucosidase–enzymes related to hyperglycemia. *Food & Function,* 4(4): 644-649.

58. Suparmi, S. & Prasetya, H. 2012. Antioxidant activity of the crude carotenoid pigment extract from yellow ambon banana (*M. parasidiaca sapientum*) peel: its potency as vitamin a supplement. *Sains Medika: Jurnal Kedokteran dan Kesehatan,* 4(1): 78-88.

59. Tang, C., Koulajian, K., Schuiki, I., Zhang, L., Desai, T., Ivovic, A., Wang, P., Robson-Doucette, C., Wheeler, M. & Minassian, B. 2012. Glucose-induced beta cell dysfunction in vivo in rats: link between oxidative stress and endoplasmic reticulum stress. *Diabetologia,* 55(5): 1366-1379.

60. Tundis, R., Menichini, F., Loizzo, M.R., Bonesi, M., Solimene, U. & Menichini, F. 2012. Studies on the potential antioxidant properties of *Senecio stabianus* Lacaita (Asteraceae) and its inhibitory activity against carbohydrate-hydrolysing enzymes. *Natural Product Research,* 26(5): 393-404.

61. Uğur, A., Ertem, H. & Beyatlı, Y. 2006. Antibacterial properties of *Senecio sandrasicus*. on multidrug-resistant *Stenotrophomonas maltophilia*. *Pharmaceutical Biology,* 44(4): 253-257.

62. Wang, H., Du, Y.J. & Song, H.C. 2010. α-Glucosidase and α-amylase inhibitory activities of guava leaves. *Food Chemistry,* 123(1): 6-13.

63. Wild, S., Roglic, G., Green, A., Sicree, R. & King, H. 2004. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care,* 27(5): 1047-1053.