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OPTIMIZATION OF PROANTOCYANIDINE EXTRACTION PROCESS FROM *FRAGARIA VESCA* L. LEAVES

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Abstract

Wild strawberries Fragaria vesca L. have been traditionally used in herbal medicine in treating rashes, as well as internally for treating gastrointestinal catarrh (mucous), diarrhea, intestinal toning, liver health maintenance, catarrh of respiratory passages, rheumatism, nervousness, bladder health maintenance, gravel, fever, in support of vascular health and as a diuretic. The leaves of Fragaria vesca L. are natural source of biologically active substance, such as condensed tannins (epigallocatechins), ellagitannins (pedunculagin and agrimoniin), flavonoids (kampferol and quercetin glucosides) and proantocyanidins (catechin, procyanidin B1). The aim of the current investigation is connected with selection of the best conditions for proanthocyanidins extraction. The influence of the duration of the ultrasonic extraction and solvent system (acetone-water) in different concentration ratio over the extraction process was studied. The optimal conditions for the extraction of proantocyanidins from strawberries leaves were as follow 56% acetone-water solvent system, time of ultrasonic extraction 50 min in ultrasonic bath with frequency 35 kHz. Under these conditions the maximum amount of proantocyanidins 124.0 mg/100 g dry biomass were obtained.

Keywords: *Fragaria vesca L., proanthocyanidins, ultrasonic extraction*

INTRODUCTION

Wild strawberry (*Fragaria vesca* L.) is a widely growing plant with medicinal properties (Figure 1). In the traditional system of medicine, the plant has been reported to possess diuretic and liver tonic properties and antimicrobial activity (Vennat et al., 1988, Kishore et al., 2012).

Figure 1. Wild strawberry (*Fragaria vesca* L.)

It has certain therapeutic application such as astringent, arthritis, diuretic, disturbances and liver tonic etc. It contains flavonoids, phenolic acids, tannins, anthocyanins, as well as anti-oxidants (Wang et al., 2000, Filippone et al., 2001).

Procyanidin dimers B1, B2, and B5 (Figure 2, 3 and 4) have been identified in *Fragaria vesca* L. (Vennat et al., 1988, 1989, Buricova et al., 2011). The biological properties of proanthocyanidins have been extensively studied.

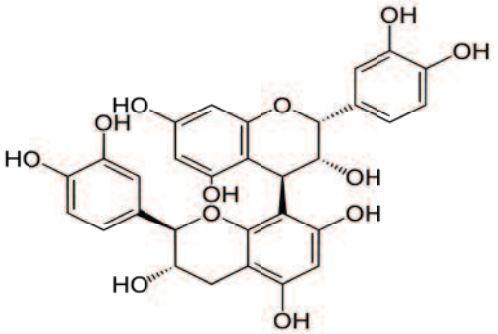

Figure 2. Procyanidine B_1 (epicatechin-(4β→8)-catechin)

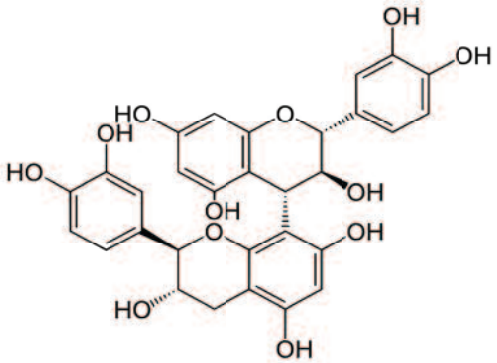

Figure 3. Procyanidine B_3 ((catechin-(4α→8)-catechin)

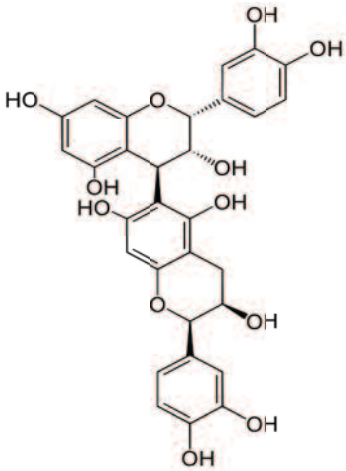

Figure 4. Procyanidine B_5 (epicatechin-(4β → 6)-epicatechin)

These substances have been possessed free radical scavenging and antioxidant activity (Dai and Mumper, 2010). Proanthocyanidins have been reported to have antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilators actions (Lin and White, 2012). They have also been shown to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A_2, cyclooxygenase, and lipoxygenase (Dai and Mumper, 2010). Procyanidines have ability non-competitively to inhibit the activity of xanthine oxidase – a major generator of free radicals, elastase, collagenase, hyaluronidase, and beta-glucuronidase (Fine, 2000).

In nowadays application of ultrasonic irradiation techniques for extraction of biologically active substances constantly increased. It was known that ultrasonic waves accelerate the diffusion process and shortened the extraction time (Lingyun et al., 2007).

On this base, the aim of our investigation was connected with selection of the best ultrasonic conditions for proanthocyanidins extraction from *Fragaria vesca* L.

MATERIALS AND METHODS

Plant material

Aerial parts (leaves) by several random chosen plants of *F. vesca* L., were collected from their natural habitats near hut "Zdravec", Rhodopa mountain all in May 2013. The samples were dried in shade at ambient temperature for 7 days, and powdered by homogenizer. The powder was used for extraction of proanthocyanidins.

Extraction procedure

Half gram dry ground leaves were placed in a plastic vial and 50 mL solvent in different ratio mixture acetone and water was poured in it. Ultrasonic extraction procedure was performed in ultrasonic bath SIEL UST 5.7-150 (Gabrovo, Bulgaria) with frequency 35 kHz and power 240 W at temperature 50°C for different time. The ratio between acetone-water and extraction time were varied in order to obtain the highest yield of proantocyanidins.

Total proanthocyanidins assay

Acid butanol assay for proanthocyanidins, according to Porter et al. (1986), was used. Six milliliters of the acid butanol reagent (950 mL of *n*-butanol with 50 mL concentrated HCl), 0.5 mL aliquot of the fraction, and 0.1 mL of the iron reagent (2 % ferric ammonium sulfate in 2 mol/L HCl) were added to 10 mL screw cap tube and then vortexed. The tube was capped loosely and put in a boiling water bath for 50 min. The absorbance of formed colored complex was read at 550 nm. Condensed tannins were analyzed as leucocyanidin equivalent (Hagerman, 2011).

Response surface methodology (RSM) was used to optimize the variables to predict the best extraction conditions. In this investigation a series of statistically designed studies were performed to reveal the effect of the independent variables (solvent ratio and extraction time). To describe the nature of response surface in optimum region a

composite design with two coded levels (X_1 – solvent acetone-water in different ratio and X_2 – extraction time) was performed. The model for predicting the optimal conditions was expressed by the following equation (1).

$$Y = b_0 + \sum b_i \ x_i + \sum b_{ii} \ x_i^2 + \sum b_{ij} \ x_i x_j \ (1)$$

Where Y is response variable; *b* is regression coefficients and x is coded levels of the independent variable. The effects of acetone-water ratio and extraction time on proanthocyanidins were also analyzed by multiple regression techniques. The predicted equation for the proanthocyanidins extraction yield (Y) was given in the following equation as a function of the coded values.

Statistical analysis was performed with Statistical Software MINITAB 16. The adequacy of model was checked accounting for R^2. Numerical optimization techniques of designed experiment were used for simultaneous optimization response.

RESULTS AND DISCUSSIONS

Many researchers worldwide have been investigated the influence of various solvents on the extraction efficiency of proanthocyanidins from different plant sources. It has been established that the most suitable solvent was acetone-water mixture (Karamac et al., 2005, Liu and White, 2012). Kajdžanoska et al. (2010) analyzed polyphenolic compounds in cultivated strawberries (*Fragaria ananassa*) fruits by HPLC–DAD-ESI-MS. However until now, no information was available for extraction of proanthocyanidines from wild strawberry leaves under ultrasonic irradiation.

One of the most successive approach for the extraction process optimization of secondary metabolites from plant materials is the statistically optimization of extraction conditions (temperature, solvent and extraction time) (Lingyun et al., 2007).

On the base of this knowledge we carried out a detailed study for the influence of different acetone–water ratio and extraction time, accompanied with ultrasonic irradiation for acceleration of the extraction procedure.

The results described above clearly outlined that the investigated variables (solvent systems and time of extraction) significantly influenced proantocyanidins extraction by *Fragaria vesca* (Table 1). The statistical regression model was obtained, taking into account the influence of the solvent (X_1) and extraction time (X_2) on the amount of total proantocyanidins yield (Y) (2).

$$Y = -119.828 + 6.981X_1 + 1.999X_2 - 0.058X_1^2 - 0.014X_2^2 - 0.010\ X_1X_2 \qquad (R^2 = 86.8) \quad (2)$$

Table 1. Independent variable values of the process and their corresponding values

	X_1 (Solvent ratio Acetone – H_2O)	**X_2 (Extraction time, min)**	**Y (Total proanthocyanidins, mg/100 g DW)**
1	30	10	58,5
2	50	10	101,3
3	70	10	84,7
4	30	20	55,3
5	50	20	120,3
6	70	20	110,1
7	30	40	76,3
8	50	40	116,0
9	70	40	121,3
10	30	60	98,6
11	50	60	112,9
12	70	60	111,0

Table 2. Comparison between theoretically calculated and experimentally obtained yields of proanthocyanidins

	X_1[1]	**X_2[1]**	**$\hat{Y}^2$max**
Theoretically calculated	55.8 %	49.8 min	125.4 mg/100 g DW
Experimentally obtained	56 %	50 min	124.0 mg/100 g DW

[1] – independent variables;

[2] – maximum yield of proanthocyanidins.

The value of obtained coefficient of determination (R^2) was good enough. The optimization procedures carried out using "Response optimizer" of MINITAB 16 software gave the following values of variable X_1 and X_2 for maximum yield of proantocyanidins (Y) by *Fragaria vesca* L. (Table 2). The deviation between the theoretically studied maximal amounts of proantocyanidins and experimentally obtained (at 56 % acetone and 50 min time of extraction) was only 1.4 mg/100 g DW under ultrasonic influence (Table 2). On this basis we propose 56% acetone in water and 50 min time of extraction as optimal for yield of proantocyanidins by *Fragaria vesca* L leaves.

CONCLUSIONS

On the base of the obtained results from process optimization of proanthocyanidins extraction we proposed ultrasonic method for accelerated extraction procedure. These optimized methods will be applied in our future investigation for extraction of procyanidins and their further addition in drug, food products and cosmetics as a source of biological activity.

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