INFLUENCE OF PROCESS PARAMETERS ON HAWTHORN (*CRATAEGUS MONOGYNA* JACK.) EXTRACTION

ORIGINAL SCIENTIFIC PAPER

Nebojša Vasiljević✉, Vladan Mićić, Ljubica Vasiljević, Dragica Lazić, Milorad Tomić, Duško Kostić

DOI: 10.51558/2232-7568.2023.16.1.29

RECEIVED 2023-08-20 **ACCEPTED** 2023-10-03

University of East Sarajevo, Faculty of Technology, Karakaj 34a, 75400 Zvornik, Bosnia and Herzegovina

✉ nebojsa.vasiljevic@tfzv.ues.rs.ba

ABSTRACT:

Given that many synthetic medications can induce a variety of negative reactions in patients, a search for natural substances with minimal side effects in patients has been conducted. Nowadays, researchers are focusing on plant medicines, which have been used to heal illnesses since ancient times. The plant *Crataegus monogyna* Jack. (hawthorn) is the most abuntant plant in the Rosaceae family that is also used in traditional medicine. *C. monogyna*'s pharmaceutical, phytochemical, functional, and therapeutic qualities are based on a wide range of useful secondary metabolites, which include phenolic compound (flavonoids, anthocyanins, tannins), vitamin C and antioxidants. Total (poly)phenols, flavonoids and anthocyanins contents in *C. monogyna* Jacq. extracts were measured using the Folin-Ciocalteu reagent, aluminium chloride and the pH differential methods, respectively. The extraction lasted 15 to 120 min, with a solid-to-solvent ratio of 1:15 w/v and 1:30 w/v and solvents of 30% and 60% ethanol. According to the results, the extraction process has the highest velocity within the first 15 min, when the majority of (poly)phenols and flavonoids are extracted, but it becomes slower as time passes. Higher yields are obtained by utilizing a solid-to-solvent ratio of 1:30 w/v rather than a solid-to-solvent ratio of 1:15 w/v, which indicates that when the amount of drug increases over a certain optimal value, the resistance to mass transfer from a solid material to liquid increases. Finally, the results about the impact of the ethanol content in the solvent demonstrate that a larger ethanol content greatly favors the extraction of flavonoids, but this is not as evident for the extraction of total (poly)phenols and anthocyanins.

KEYWORDS: anthocyanins, extraction, flavonoids, (poly)phenols

INTRODUCTION

It is well known that the usage of medicinal herbs is a significant factor that improves people's overall health, and the study of its positive influence is becoming an increasingly popular topic of contemporary research.

Medicinal plants are rich in (poly)phenolic compounds and as such represent a good antioxidant agent. One of a such medicinal plants is hawthorn. The Hawthorn species belongs to the Rosaceae family and the genus *Crataegus* [1]. The Rosaceae family's most significant genus, *Crataegus L.*, is thought to have between 150 and 1200 species, depending on the species concept used and the insertion of several plausible taxonomic hybrid origins [2]. Even though the taxonomy of the genus is quite complicated and, in the past, the species name *C. oxyacantha L.* (and *C. oxyacantha* Jacq. and other names) was used, the common hawthorn is primarily referred to as *C. monogyna* Jacq [3].

Hawthorn is a semi-evergreen shrub or small tree with thorns that may grow up to 5-15 m [4]. Leaf shape, seed characteristics, seed quantity, and fruit color are all very variable. The bark is smooth grey, with shallow longitudinal cracks and small ridges in the older phenological stage. Each flower has five to twenty-five anthers, five sepals, and five petals. The petals are white and pink, and they are frequently longer than the sepals. The Hawthorn fruit is normally fleshy and can be yellowish, reddish, or blackish-purple. Each fruit contains one to five solid seeds [3], [4].

Herbal drugs are made from the leaves, flowers, and fruits of the hawthorn. Flowers are harvested early in the spring when they are fully developed, leaves in the summer, and fruits in the middle of the fall when they are ready. Complexes of flavonoid heterosides in the amount of 1-2% are found in hawthorn flower and leaf. Fruits include fewer flavonoids (approximately 0.1%) but more sugars, organic acids, carotenoids, and vitamin C [5].

The high content of phenolic compounds such as flavonoids, proanthocyanidins, catechins, phenolic acids, essential oils, and terpenoids explain the use of hawthorn extract as natural therapy for a variety of pharmacological diseases [6]-[9]. Furthermore, some studies claim that carotenoids have an important antioxidant function in plants by deactivating singlet oxygen, which is formed during photosynthesis [10]. Hawthorn also has tonic effects on the heart [8], and multiple studies have shown that it can decrease a variety of cardiovascular risk factors such as hypertension, hypercholesterolaemia, and so on [11], [12].

Various solvents, including methanol, ethanol, acetone, and ethyl acetate, as well as their mixtures, have been employed to extract (poly)phenolic compounds from fresh, frozen, or dried plant material [13]. The type and amount of extracted (poly)phenolic compounds are influenced by the extraction solvent concentration, solid-to-solvent ratio, temperature and duration of extraction process. Methanol and methanol-water mixes are the most often used solvents for phenolics extraction, however ethanol and ethanol-water mixtures also yield good results. Some authors recommend using ethanol as a solvent for phenolic extraction since it is non-toxic and allows them to alter the ethanol to water ratio to modify the polarity of the mixture [14], [15].

MATERIAL AND METHODS

PLANT MATERIALS AND REAGENTS

Fresh hawthorn leaves and flowers, harvested in spring, was used for the extraction. Ethanol was used for sample extraction, while extract characterization was performed using the following reagents: Folin-Ciocalteu reagent (Carlo Erba, Germany), sodium carbonate (Lach:ner, Czech Republic), gallic acid (Sigma Aldrich, USA), aluminum chloride (Lach:ner, Czech Republic), sodium hydroxide (Lach:ner, Czech Republic), sodium nitrite (Zorka Šabac, Serbia), catechin hydrate (Sigma Aldrich, USA), and potassium chloride buffer pH=1.0 (Lach:ner, Czech Republic).

EXTRACTION

The traditional maceration technique was utilized in this study to extract (poly)phenolic compounds from the drug material. The experiment was carried out at room temperature in laboratory beakers containing the plant and the solvent. Occasional mixing ensures that the plant is in contact with the solvent, which promotes extraction.

The maceration procedure was performed under the following process conditions:

- Extraction time [min] – 15, 30, 45, 60 and 120,
- Percentage of ethanol in the extraction solvent [vol. %] – 30 and 60 and
- Solid-to-solvent ratio [w/v] – 1:15 and 1:30.

The influence of previously mentioned process parameters on the yield of total (poly)phenols, flavonoids, and anthocyanins in extracts was examined in this paper.

METHODS

Determination of total (poly)phenols content is based on oxidation-reduction reactions involving hydroxyl groups of phenol and the Folin-Ciocalteu reagent, as well as polymer complex ions of molybdenum and tungsten. The reaction requires a basic environment, which is created by adding sodium carbonate to the reaction mixture. The measurement is spectrophotometric, at wavelength of 765 nm, and gallic acid is utilized as the standard [16]. A Shimadzu 1800 spectrophotometer was utilized for spectrophotometric determination, with the calibration curve ranging from 50 to 500 mg/l of gallic acid. The results are given in milligrams of gallic acid equivalent per gram of plant material (mg GAE/g).

The flavonoids content of the sample is determined using the colorimetric technique with aluminum chloride. In an acidic solution, aluminum chloride forms stable complexes with the C-4 keto group or the C-3 and C-5 hydroxyl groups of the present flavones and flavonols, and unstable complexes with orthodihydroxyl groups in the A or B ring of flavonoids. Measurement is spectrophotometric at wavelenght of 510 nm, with catechin hydrate as the standard [17]. For determination of flavonoids the calibration curve was in range 20 to 200 mg/l of catechin hydrate. The results are given in milligrams of catechin hydrate equivalents per gram of plant material (mg CTH/g).

The quantitative determination of total anthocyanins (non-degraded monomers and products of their degradation) is based on the property of anthocyanins to reversibly change their structure when the pH of the environment changes, which also changes the absorption spectrum. The content of total anthocyanins is determined by the "single" method, described in the paper [18], which is based on measuring the absorbance of the anthocyanin solution at pH=1.

The total anthocyanins concentration in the sample is determined as cyanidin-3-glucoside equivalent (mg Cy3G/g) using the formula:

$$C_{tot} = (A \cdot M \cdot F \cdot 10^3)/(\varepsilon \cdot l \cdot R) \quad (1)$$

where are:
C_{tot} – total anthocyanins content,
$A = (A_{520nm} - A_{700nm})_{pH=1,0}$,
M = molar mass (for Cy3G it is 449,2 g/mol),
F = dilution factor,
10^3 = factor for converting grams to miligrams,
ε = molar absorption extinction coefficient (for Cy3G it is 26900 $Lmol^{-1}$ cm^{-1})
l = cuvette thickness (1 cm) and
R – factor for recalculating the value of anthocyanins per gram of drug.
A Shimadzu 1800 spectrophotometer was used to determine anthocyanins, same as it was for total (poly)phenols and flavonoids.

STATISTICAL ANALYSIS

Statistical analysis was performed using MINITAB 21. Given that a high rate of extraction is anticipated at first, followed by a gradual slowing [19], a concave function was employed to approximate the experimental data. The formula for that function is as follows:

$$Y = a \cdot X/(b + X) \quad (2)$$

where are:
Y – (Poly)phenols (flavonoids or anthocyanins) content [mg/g],
X – Time [min] and
a, b – coefficients.

By choosing a confidence level of 95%, the coefficients a and b in the previous concave function were determined. The algorithm used is Gauss-Newton, the maximum number of iterations is 200, while the tolerance is 0.00001.

RESULTS AND DISCUSSION

Figure 1 depicts the time dependence of extracted (poly)phenols under constant other process parameters (solid-to-solvent ratio and percentage of ethanol in the extraction solvent). Since the parameters solid-to-solvent ratio and percentage of ethanol in the extraction solvent have two levels per experimental design setup, a total of 2^2=4 combinations will be utilized to illustrate the time dependency of total (poly)phenolic yield.

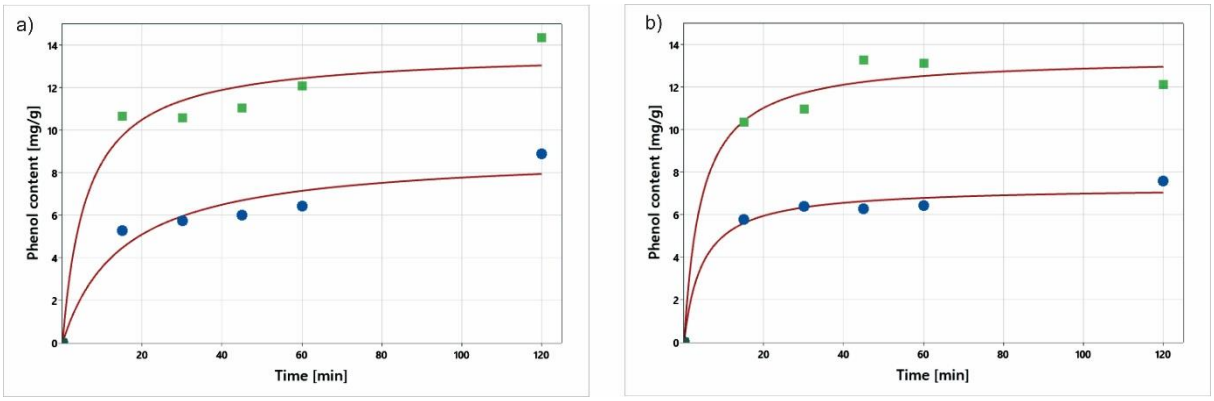

Figure 1. Effect of extraction time on the content of extracted total (poly)phenols with a) 30% ethanol and b) 60% ethanol (• - solid-to-solvent ratio of 1:15 w/v and ▪ - solid-to-solvent ratio of 1:30 w/v)

According to the findings, time has a substantial influence on the extraction of (poly)phenolic compounds from the hawthorn. There are two extraction periods: a fast extraction period (during the first 15 min of extraction) in which (poly)phenolic compounds are extracted intensely from the drug, and a slow extraction period in which the rate of extraction is significantly slower. These findings are consistent with previous research on the extraction of phenols from medicinal plants [20].

However, when the extraction periods are examined independently, it is discovered that parameters solid-to-solvent ratio and percentage of ethanol in the extraction solvent have different effects on the extraction rate. When the solid-to-solvent ratio is 1:15 w/v and 30% ethanol is used (lower extraction conditions), the extraction is slower in the beginning, and after 15 min, only 5.27 mg GAE/g of (poly)phenols are extracted. However, there is a further rise in (poly)phenol in the extract during the second period, with the amount present increasing by 40.7% (to a value of 8.9 mg GAE/g). The application of a solid-to-solvent ratio of 1:30 w/v and 60% ethanol (higher extraction parameters) have the opposite effect. During a fast extraction period, 10.37 mg GAE/g of (poly)phenols was extracted from hawthorn, but with further prolongation of the extraction time, the (poly)phenols content increased to 12.15 mg GAE/g, representing a 14.7% increase. So, when lower extraction parameters are used, the extraction

velocity is low at first but steadily increases during the second extraction period, but when higher extraction parameters are used, the extraction velocity is high at first but relatively unchanged during the second extraction period.

There are a similar increase in the second extraction period with the solid-to-solvent of 1:30 w/v and the use of 30% ethanol and with the solid-to-solvent ratio of 1:15 w/v and the use of 60% ethanol; in the first case, there is an increase from 10.68 mg GAE/g to 14.35 mg GAE/g (which represents a 25.5% increase), while in the second case, there is an increase from 5.79 mg GAE/g to 7.58 mg GAE/g (which represents an increase in the amount of 23.6%).

Observing the parameter percentage of ethanol in the extraction solvent, for example, after 120 min of extraction and with a solid-to-solvent ratio of 1:30 w/v, it is observed that the content of (poly)phenols (12.15 mg GAE/g) with 60% ethanol is slightly lower than the content of (poly)phenols with 30% ethanol (14.35 mg GAE/g). Therefore, increasing the ethanol content in the solvent above 30% reduces the yield of (poly)phenols. Such behavior was noticed by other authors [21].

The solid-to-liquid ratio, on the other hand, has major effects on the yield of (poly)phenols. For example, after 120 min of extraction and 30% alcohol, the (poly)phenols content is 8.9 mg GAE/g with a solid-to-solvent ratio of 1:15 w/v, and 14.35 mg GAE/g with a solid-to-solvent ratio of 1:30 w/v. Similarly, after 120 min of extraction and the use of 60% ethanol, the level of (poly)phenols is 7.58 mg GAE/g with a solid-to-solvent ratio of 1:15, and 12.15 mg GAE/g with a solid-to-solvent ratio of 1:30 w/v. Based on the previous results, better yields of (poly)phenols per unit mass are obtained at larger solid-to-solvent ratios. These results were consistent with mass transfer principles where the driving force for mass transfer is considered to be the concentration gradient between the solid and the solvent [15], [22]. Higher solid-to-solvent ratio increases the concentration gradient, leading to an increased diffusion rate of the compounds from the extracted solid material into the solvent, but also determines the increasing of the necessary period of time to achieve equilibrium. Solid-to-solvent ratio could significantly affect the equilibrium constant and characterize the relationship between yield and solvent use as a steep exponential increase followed by a steady state to give the maximum yield [15], [23].

The statistical program MINITAB 21 was used to determine the regression equation for the dependence of the (poly)phenols yield in the extract on the extraction time. The results of the statistical analysis are shown in Table 1.

Table 1. Determination of coefficients a and b in regression equations of (poly)phenols content dependency on time

Constant values	Summary		Parameter	Estimate	SE[a] Estimate	95% Cl[b]
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations	14	a	8.9463	1.11727	(6.29654, 14.3942)
	Final SSE[c]	2.57136	b	14.9691	7.18346	(0.43926, 58.4699)
	DFE[d]	4				
	MSE[e]	0.642840				
	S[f]	0.801773				
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations	10	a	13.7233	1.06688	(10.9998, 17.6454)
	Final SSE	4.33319	b	6.1009	3.18328	(-1.0474, 20.1872)
	DFE	4				
	MSE	1.08330				
	S	1.04082				
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations	9	a	7.31804	0.37160	(6.34958, 8.5092)
	Final SSE	0.594733	b	4.56873	1.92009	(0.00023, 11.5076)
	DFE	4				
	MSE	0.148683				
	S	0.385595				
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations	8	a	13.4421	0.78474	(11.4795, 15.8545)
	Final SSE	2.70162	b	4.3462	2.18051	(-0.5118, 11.8669)
	DFE	4				
	MSE	0.675406				
	S	0.821831				

where are:
[a]SE – standard error
[b]95% Cl - The 95% Confidence Interval
[c]SSE – Sum of Squares for Error
[d]DFE - Degrees of Freedom for Error
[e]MSE – Mean Squared Error
[f]S – Standard Deviation

The coefficients of the regression equation and the divergence of the actual from the theoretical values were estimated based on data processing in MINITAB 21.

The regression equations of the dependence of the total (poly)phenols content in the extract on the extraction time (0-120 min) have the following form:

1. Solid-to-solvent ratio of 1:15 w/v and 30% ethanol
$Y = 8.94628 \cdot X / (14.9691 + X)$
2. Solid-to-solvent ratio of 1:30 w/v and 30% ethanol
$Y = 13.7233 \cdot X / (6.10089 + X)$
3. Solid-to-solvent ratio of 1:15 w/v and 60% ethanol
$Y = 7.31804 \cdot X / (4.56873 + X)$
4. Solid-to-solvent ratio of 1:30 w/v and 60% ethanol
$Y = 13.4421 \cdot X / (4.34617 + X)$

where are:
Y – (Poly)phenols content [mgGAE/g] and
X – Time [min].

Figure 2 depicts the time dependence of extracted flavonoids under constant other process parameters (solid-to-solvent ratio and percentage of ethanol in the extraction solvent). As with content of (poly)phenols, a total of 2^2=4 combinations will be utilized to illustrate the time dependency of flavonoids yield.

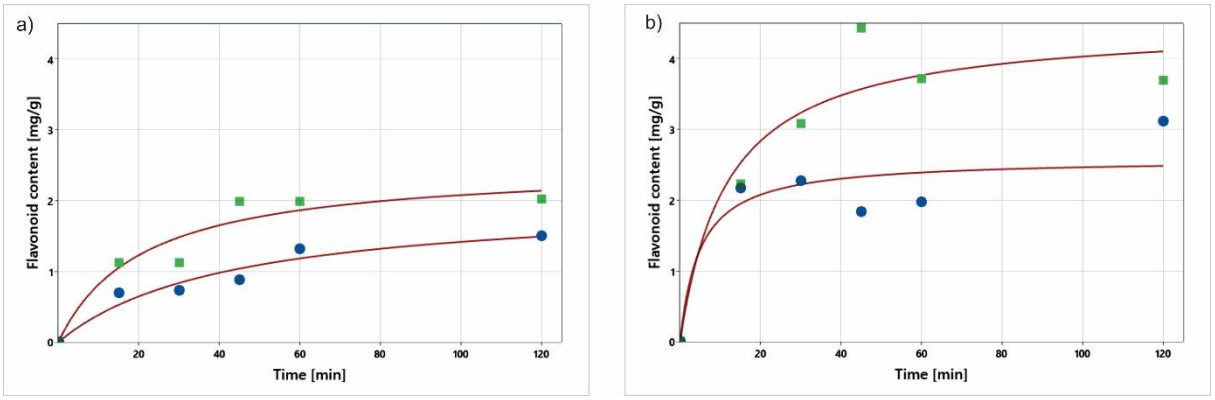

Figure 2. Effect of extraction time on the content of extracted flavonoids with a) 30% ethanol and b) 60% ethanol (• - solid-to-solvent ratio of 1:15 w/v and ▪ - solid-to-solvent ratio of 1:30 w/v)

Based on Figure 2, it can be concluded that the content of flavonoids is aproximetely 3-6 times smaller then the content of (poly)phenols, depends on other process condition.

As with extraction of total (poly)phenols, we also here differentiate two extraction periods: initial (fast extraction period) extraction and steady (slow extraction period).

The increase in the yield of flavonoids in the extract during the period of slow extraction is similar for all four cases: with a solid-to-solvent ratio of 1:15 w/v and 30% ethanol there is an increase in the content of flavonoids from 0.7 mg CTH/g to 1.5 mg CTH/g, which represents an increase of 53.3%, with a solid-to-solvent ratio of 1:30 w/v and 30% ethanol there is an increase of 44.6% (from 1.12 mg CTH/g to 2.02 mg CTH/g), with a solid-to-solvent ratio of 1:15 w/v and 60% ethanol there is an increase in the amount of 30.1% (from 2.18 mg CTH/g to 3.12 mg CTH/g), while with a solid-to-solvent ratio of 1:30 w/v and 60% ethanol there is an increase of 39.5% (from 2.24 mg CTH/g to 3.7 mg CTH/g). Therefore, the extraction of flavonoids is somewhat slower in the initial extraction period, but the extraction velocity does not decrease drastically in the second period.

When the solid-to-solvent ratio is examined in relation to flavonoids yield, it is discovered that as this ratio grows, so does the yield. As an example, after 120 min of extraction with 30% ethanol, the extracted flavonoids content is 1.5 mg CTH/g at a solid:liquid ratio of 1:15 w/v, and 2.02 mg CTH/g at a solid-to-solvent ratio of 1:30 w/v. When 60% ethanol is used, the same response is observed. As a result, as with (poly)phenols extraction, using a smaller amounts of the drug results in a better yield per unit mass of the drug.

On the other hand, observing the influence of the ethanol content in the solvent on the yield of flavonoids, an interesting effect is observed. For example, after 120 min of extraction and a solid-to-solvent ratio of 1:15 w/v, using 30% ethanol the yield of flavonoids is 1.5 mg CTH/g, while using 60% ethanol the yield is 3.12 mg CTH/g. Likewise, at the same time of extraction but with a solid-to-solvent ration of 1:30 w/v, using 30% ethanol, the flavonoids content is 2.02 mg CTH/g, and using 60% ethanol, this content is 3.7 mg CTH/g. Thus, the yield of flavonoids increases twice with the use of a solvent containing 60% ethanol compared to a solvent containing 30% ethanol.

The regression equation for the dependency of the yield of flavonoids in the extract on the extraction time was determined using the statistical application MINITAB 21. Table 2 displays the statistical analysis findings.

Table 2: Determination of coefficients a and b in regression equations of flavonoid content depending on time

Constant values	Summary		Parameter	Estimate	SE Estimate	95% CI
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations	8	a	2.0195	0.3853	(1.24213, 4.355)
	Final SSE	0.0853752	b	42.3903	18.9276	(8.89972, 175.697)
	DFE	4				
	MSE	0.0213438				
	S	0.146095				
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations	7	a	2.5127	0.4036	(1.67868, 4.2524)
	Final SSE	0.240507	b	20.8372	10.8189	(2.21770, 77.0574)
	DFE	4				
	MSE	0.0601268				
	S	0.245208				
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations	10	a	2.58846	0.45598	(1.54698, 5.8812)
	Final SSE	0.871403	b	4.90151	6.78238	(-6.65384, 83.2469)
	DFE	4				
	MSE	0.217851				
	S	0.466745				
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations	11	a	4.5078	0.63293	(3.17555, 6.7978)
	Final SSE	1.01243	b	11.7599	7.27309	(-0.57881, 43.8438)
	DFE	4				
	MSE	0.253108				
	S	0.503098				

The coefficients of the regression equation and the divergence of the actual from the theoretical values were estimated based on data processing in MINITAB 21.

The regression equations of the dependence of the flavonoids content in the extract on the extraction time (0-120 min) have the following form:

1. Solid-to-solvent ratio of 1:15 w/v and 30% ethanol
$Y = 2.0195 \cdot X / (42.3903 + X)$
2. Solid-to-solvent ratio of 1:30 w/v and 30% ethanol
$Y = 2.5127 \cdot X / (20.8372 + X)$
3. Solid-to-solvent ratio of 1:15 w/v and 60% ethanol
$Y = 2.58846 \cdot X / (4.90151 + X)$
4. Solid-to-solvent ratio of 1:30 w/v and 60% ethanol
$Y = 4.5078 \cdot X / (11.7599 + X)$

where are:
Y - Flavonoids content [mg CTH/g] and
X – Time [min].

The dependence of anthocyanins content in the extract on time under constant other process parameters (solid-to-solvent ratio and percentage of ethanol in the extraction solvent) is shown in Figure 3. As in previous cases, a total of 2^2=4 combinations will be utilized to illustrate the time dependency of anthocyanins yield.

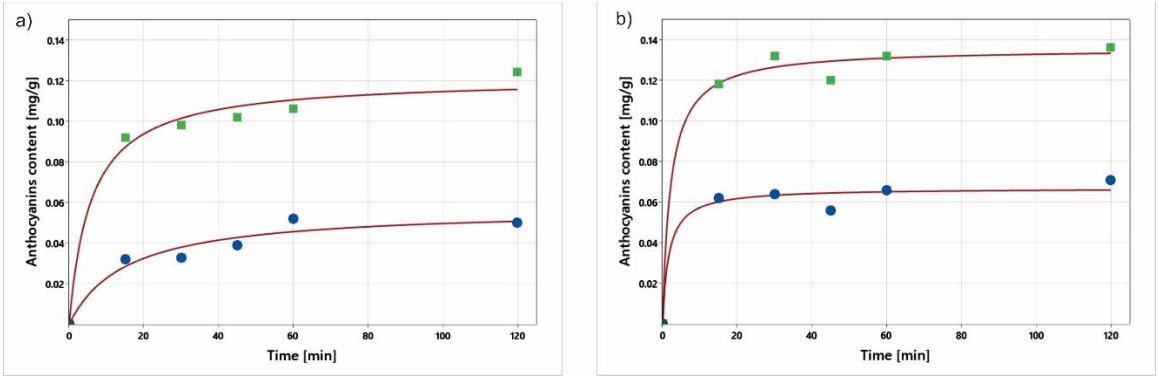

Figure 3. Effect of extraction time on the content of extracted anthocyanins with a) 30% ethanol and b) 60% ethanol (• - solid-to-solvent ratio of 1:15 w/v and ▪ - solid-to-solvent ratio of 1:30 w/v)

The findings and conclusions are similar to those for (poly)phenols and flavonoids: there is a period of fast extraction (the first 15 min) followed by a period of slow extraction. Examining through each of the diagrams individually, it can be concluded that the other process parameters have different effects on these extraction periods; in the period of slow extraction with lower extraction parameters (solid-to-solvent ratio of 1:15 w/v and usage of 30% ethanol), there are a 36% increase in anthocyanins content (from 0.032 mg Cy3G/g to 0.050 mg Cy3G/g), while at higher extraction parameters (solid-to-solvent ratio of 1:30 w/v and usage of 60% ethanol), there are a 13.2% increase from 0.118 mg Cy3G/g to 0.136 mg Cy3G/g).

Observing the effect of the solid-to-solvent ratio on anthocyanins content in the extract, it is discovered that increasing the ratio from 1:15 w/v to 1:30 w/v improves the anthocyanins content by 2-3 times, depending on other process parameters. For example, using 30% ethanol and after 15 min of extraction, at a solid-to-solvent ratio of 1:15 w/v the anthocyanins content is only 0.032 mg Cy3G/g, while at a solid-to-solvent ratio of 1:30 w/v the anthocyanins content is even three times higher and amounts to 0.092 mg Cy3G/g). If the amount of drug is more than optimal, it can be concluded that resistance to mass transfer exists in this situation as well.

Concerning the influence of percentage of ethanol in the extraction solvent, an increase in its content results in a rise in yield of anthocyanins, but it doesn't have as much impact as a solid-to-solvent ratio. For example, after 120 min of extraction and at a solid-to-solvent ratio of 1:30 w/v, using the 30% ethanol the anthocyanins content is 0.124 mg Cy3G/g, while using the 60% ethanol the anthocyanins content is slightly larger and amounts 0.136 mg Cy3G/g)

Observing some absolute values of anthocyanins content in the extract, e.g. after 120 min, it is observed that they range from 0.05 to 0.136 mg Cy3G/g; therefore, the content of anthocyanins in ethanole extracts of hawthorn is much smaller in comparison to the overall quantity of (poly)phenols in the ethanol extracts.

The results of the statistical analysis determined in the statistical program MINITAB 21 are shown in Table 3.

Table 3. Determination of coefficients a and b in the regression equations of the dependence of anthocyanins content in the extract on time

Constant values	Summary		Parameter	Estimate	SE[a] Estimate	95% CI[b]
Solid-to-solvent ratio of 1:15 and 30% ethanol	Iterations	6	a	0.0573	0.00675	(0.04177, 0.0844)
	Final SSE[c]	0.0000926	b	15.1665	6.81291	(1.71307, 47.8126)
	DFE[d]	4				
	MSE[e]	0.0000232				
	S[f]	0.0048122				
Solid-to-solvent ratio of 1:30 and 30% ethanol	Iterations	9	a	0.12149	0.00630	(0.105001, 0.1421)
	Final SSE	0.0001538	b	5.87788	2.10012	(0.838620, 13.6339)
	DFE	4				
	MSE	0.0000385				
	S	0.0062010				
Solid-to-solvent ratio of 1:15 and 60% ethanol	Iterations	8	a	0.06701	0.00431	(0.05591, 0.08110)
	Final SSE	0.0001024	b	1.67812	2.03425	(-2.95444, 9.54932)
	DFE	4				
	MSE	0.0000256				
	S	0.0050596				
Solid-to-solvent ratio of 1:30 and 60% ethanol	Iterations	7	a	0.13588	0.00493	(0.122916, 0.15056)
	Final SSE	0.0001283	b	2.19390	1.18955	(-0.706813, 6.04411)
	DFE	4				
	MSE	0.0000321				
	S	0.0056625				

Based on the data processing in MINITAB 21, the coefficients of the regression equation were obtained and the deviation of the actual from the theoretical values was also calculated.

The regression equations of the dependence of the anthocyanins content in the extract on the extraction time (0-120 min) have the following form:

1. Solid-to-solvent ratio of 1:15 w/v and 30% ethanol

$Y = 0.0573 \cdot X / (15.1665 + X)$

2. Solid-to-solvent ratio of 1:30 w/v and 30% ethanol

$Y = 0.12149 \cdot X / (5.87788 + X)$

3. Solid-to-solvent ratio of 1:15 w/v and 60% ethanol

$Y = 0.06701 \cdot X / (1.67812 + X)$

4. Solid-to-solvent ratio of 1:30 w/v and 60% ethanol

$Y = 0.13588 \cdot X / (2.19390 + X)$

where are:

Y - Anthocyanins content [mg Cy3G/g] and

X – Time [min].

CONCLUSION

The effect of process parameters (time, solid-to-solvent ratio, and percentage of ethanol in the extraction solvent) on the yield of (poly)phenolic chemicals in howthorn (*Crataegus Monogyna* Jack.) extract was investigated in this paper. In terms of time as a process parameter, it was established that there are two separate extraction periods: a fast extraction period, which occurs during the first 15 min, and a slow extraction period (after 15 min). By comparing the effect of different ethanol content in the solvent on the yield, it was determined that a higher ethanol content in the solvent enables better extraction only for flavonoids and anthocyanins, but not for total (poly)phenols. Higher solid-to-solvent ratios contribute to more effective extraction of all (poly)phenolic components due to easier diffusion into the liquid in the presence of a smaller amount of drug relative to the liquid.

When the content of anthocyanins in the extract is compared to the overall polyphenol content, it may be determined that just a small amount of the polyphenols is anthocyanins. By comparing extraction with different percentage of ethanol in the extraction solvent and the solid-to-solvent ratio, it was discovered that a higher percentage of ethanol in the solvent and a higher solid-to-solvent ratio promote anthocyanins extraction.

REFERENCES

[1] M. Gundogdu, K. Ozrenk, S. Ercisli, T. Kan, O. Kodad and A. Hegedus, "Organic acids, sugars, vitamin C content and some pomological characteristics of eleven hawthorn species (Crataegus spp.) from Turkey," *Biological Reasearch,* vol. 47, pp. 1-5, 2014.

[2] E. Attard and H. Attard, "Hawthorn: *Crataegus oxyacantha, Crataegus monogyna* and related species," in *Nonvitamin and Nonmineral Nutritional Supplements*, Malta, Division of Rural Sciences and Food Systems, Institute of Earth Systems, University of Malta, Msida, 2019, pp. 289-293.

[3] F. Martinelli, A. Perrone, S. Martinelli, A. Papini, S. Castiglione, F. Guarino, A. Cicatelli, M. Aelaei, N. Arad, M. Gholami and S. Alireza Salami, "Botanical, phytochemical, anti-microbial and pharmaceutical characteristics of hawthorn (*Crataegus monogyna* Jacq.), Rosaceae," *Molecules,* vol. 26, no. 23, p. 7266, 2021.

[4] W. Benabderrahmane, M. Lores, O. Benaissa, J. Lamas, T. de Miguel, A. Amrani, F. Benayache and S. Benayache, "Polyphenolic content and bioactivities of *Crataegus oxyacantha L.* (Rosaceae)," *Natural Product Research,* vol. 35, pp. 627-632, 2021.

[5] V. Dekić, N. Ristić, B. Dekić and M. Ristić, "Phenolic and flavonoid content and antioxidant evaluation of hawthorn (*Crataegus monogyna* Jacq.) fruits and leaves extracts," *The University Thought - Publication in Natural Sciences,* vol. 10, no. 1, pp. 20-25, 2020.

[6] J. Edwards, P. Brown, N. Talent, T. Dickinson and P. Shipley, "A review of the chemistry of the genus Crataegus," *Phytochemistry,* vol. 79, pp. 5-26, 2012.

[7] R. García-Mateos, E. Ibarra-Estrada and R. Nieto-Angel, "Antioxidant compounds in hawthorn fruits (Crataegus spp.) of Mexico," *Revista Mexicana de Biodiversidad,* vol. 84, pp. 1298-1304, 2013.

[8] T. Bahorun, E. Aumjaud, H. Ramphul, M. Rycha, A. Luximon-Ramma, F. Trotin and O. Aruoma, "Phenolic constituents and antioxidant capacities of *Crataegus monogyna* (Hawthorn) callus extracts," *Food / Nahrung,* vol. 47, pp. 191-198, 2003.

[9] A. Kirakosyan, E. Seymour, P. Kaufman, S. Warber, S. Bolling and S. Chang, "Antioxidant capacity of polyphenolic extracts from leaves of *Crataegus laevigata* and *Crataegus monogyna* (hawthorn) subjected to drought and cold stress," *Journal of Agricultural and Food Chemistry,* vol. 71, p. 3973–3976, 2003.

[10] Pečar and V. Doleček, "Densities of beta-carotene-supercritical carbon dioxide mixtures," *Journal of Chemical & Engineering Data,* vol. 52, pp. 2442-2445, 2007.

[11] W. T. Chang, J. Dao and Z. Shao, "Hawthorn: Potential Roles in Cardiovascular Disease," *The American Journal of Chinese Medicine,* vol. 33, pp. 1-10, 2005.

[12] S. Salehi, S. Long, P. Proteau and T. Filtz, "Hawthorn (*Crataegus monogyna* Jacq.) extract exhibits atropine-sensitive activity in a cultured cardiomyocyte assay," *Journal of Natural Medicines,* vol. 63, pp. 1-8, 2009.

[13] J. Dai and R. Mumper, "Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties," *Molecules,* vol. 15, no. 10, pp. 7313-7352, 2010.

[14] D. Franco, J. Sineiro, M. Rubilar, M. Sanches, M. Jerez, M. Pinelo, N. Costoya and M. Nunes, "Polyphenols from plant materials: Extraction and antioxidant power," *Electronic Journal of Environmental, Agricultural and Food Chemistry,* vol. 7, no. 8, pp. 3210-3216, 2008.

[15] N. C. Predescu, C. Papuc, V. Nicorescu, I. Gajaila, G. Goran, C. Petcu and G. Stefan, "Polyphenolic content and antioxidantactivity of hawthorn (*Crataegus monogyna*) alcoholic extracts depending on solid-to-solvent ratio and extraction method," in *7th International Conference on Polyphenols and Health (ICPH 2015)*, Tours, Franţa, 2016.

[16] International Organization for Standardization, "ISO 14502-1:2005 Determination of substances characteristic of green and black tea — Part 1: Content of total polyphenols in tea — Colorimetric method using Folin-Ciocalteu reagent," 2005.

[17] P. Smolinski-Savi, L. Dos Santos, A. Goncalves, S. Biesek and C. Lima, "Analysis of total flavonoids present in some of the most consumed conventional and organic fruits and vegetables in southern Brazil," *Demetra,* pp. 275-288, 2017.

[18] J.M. Veličković, "Hemijska analiza i antioksidativna aktivnost odabranih biljnih vrsta bogatih fenolnim jedinjenjima – doktrorska disertacija" *Univerzitet u Nišu, Prirodno – matematički fakultet*, Niš, 2013.

[19] G. Lopez, M. Brousse and A. Linares, "Kinetic modelling of total phenolic compounds from *Ilex paraguariensis* (St. Hil.) leaves: Conventional and ultrasound assisted extraction," *Food and Bioproducts Processing,* vol. 139, pp. 75-88, 2023.

[20] A. Jovanović, V. Đorđević, G. Zdunić, D. Pljevljakušić, K. Šavikin, D. Gođevac and B. Bugarski, "Optimization of the extraction process of polyphenols from *Thymus serpyllum L,*

herb using maceration, heat- and ultrasound-assisted extraction," *Separation and Purification Technology*, vol. 179, pp. 369-380, 2017.

[21] S. Jokić, A. Bucić-Kojić, M. Planinić, D. Velić, S. Tomas, M. Bilić and Ž. Bežvir, "The effect of solvent and temperature on extraction yield of phenolic compounds from soybeans, antioxidant activity and colour of extracts," in *5th International Congress FLOUR-BREAD '09*, Opatia, Croatia, 2009.

[22] P. Tan, C. Tan and C. Ho, "Antioxidant properties: Effects of solid-to-solvent ratio on antioxidant compounds and capacities of Pegaga (*Centella asiatica*)," *International Food Research Journal*, vol. 18, pp. 557-562, 2011.

[23] S. Hamdan, H. Daood, M. Toth-Markus and V. Illes, "Extraction of cardamom oil by supercritical carbon dioxide and sub-critical propane," *The Journal of Supercritical Fluids*, vol. 44, no. 1, pp. 25-30, 2008.

THIS PAGE OF
TECHNOLOGICA ACTA
INTENTIONALLY LEFT BLANK