THE EFFECT IF EXTRACTION TIME AND SAMPLE RATIO ON THE PROCESS OF EXTRACTING ACTIVE SUBSTANCES FROM HAWTHORN *(CRATAEGUS MONOGYNA)* USING ULTRASOUND

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Abstract

Ultrasound-assisted extraction (UAE) is one of the modern method that is applied today with the aim to decrease solvent consumption, shorten the extraction time, increase the extraction yield, and enhance the quality of extracts. In this paper, the effect of extraction time and the ratio of the sample to the solvent have been studied on the ultrasonic extraction of hawthorn fruits. The hawthorn fruit was collected from Normafa, Hungary. 10% ethanol and pure distilled water were used as extraction solvent. Folin-Ciocalteu method was performed to visualization of total phenolic compounds (TPC) and the ferric reduction antioxidant power (FRAP) method to quantify antioxidant activity in the extracts. Analyzing the results by two-way-ANOVA at significant level $p < 0.05$ by using SPSS software demonstrated that there is significant effect of time of extraction and the ratio of the sample to solvent.

For TPC the best value was obtained at (9 min, 0.05 g/ml sample-solvent ratio) with a mean (451.106 ± 0.79 mg/L) by using 10% ethanol, and at (3 min, 0.075 g/ml sample-solvent ratio) with a mean (365.289 ± 15.96 mg/L) by using distilled water. While the best antioxidant activity was with a mean (223.033 ± 1.01 mg/L) at (9 min, 0.075 g/ml sample-solvent ratio) and (50.678 ± 2.02 mg/L) at (6 min, 0.1 g/ml sample-solvent ratio) by using the 10% ethanol and distilled water respectively. More work is needed to define better conditions for using the distilled water as a solvent in the ultrasonic extraction.

Introduction

Hawthorn is a common name of all plant species in the genus Crataegus, that is belonging to the Rosaceae family, the size of the trees ranged between shrubs and trees that can reach a height of up to 10 m. Normally has bright green leaves, white flowers, and bright red berries, each containing one to three or five seeds, depending on the species. Most of the species grow in North America as well as in East Asia, Europe especially Turkey [1,2]. The fruits of hawthorns are also edible, as most people who have tried them will testify. The colour of the ripe fruit ranges from yellow, through green to red, and on to dark purple. Most of the species ripen their fruit in early to mid-autumn [3]. The leaves, flowers, and berries of hawthorn contain a variety of flavonoids which include oligomeric procyanidins, vitexin, quercetin, and hyperoside. Other chemical constituents include tannins, nitrogen-containing compounds, triterpenoids such as ursolic acid, 2α,3β,19α-trihydroxyursolic acid, corosolic acid, cycloartenol, uvaol, oleanolic acid, crataegolic acid, butyrospermol, 24-methylene-24-dihydrolanosterol. Table 1 is showed the chemical constituents of hawthorn fruits [4].

Table 1: chemical constituents of hawthorn fruits.

Chemical constituents	The amount	Reference
Flavonoids	0.1- 1 %	[1]
Oligomeric proanthocyanidins	1-3%	
Triterpene acids	0.5-1.4%	
Organic acids	2-6%	
Epicatechin	178.3 mg/ 100g dry fruit	[5]
Chlorogenic acid	69.4 mg/ 100g dry fruit	
Hyperoside	24.6 mg/ 100g dry fruit	
Isoquercitrin	13.4 mg/ 100g dry fruit	
Protocatechuic acid	3.2 mg/ 100g dry fruit	
Rutin	2.6 mg/ 100g dry fruit	
Quercetin	0.9 mg/ 100g dry fruit	

In folk medicine, hawthorn fruits have been used as a cure against stress, nervousness, sleep disorders, heartache, stomachache, and sore throat [6]. Nowadays hawthorn extracts are among the most popular herbal medicinal products in many European countries and the USA after getting attention due to its potential cardiovascular enhancing and protective properties, where In Germany, hawthorn extract has been approved and registered as a therapeutic agent for the treatment of minor forms of coronary heart disease and congestive heart failure. The current study suggested that hawthorn could be used as an alternative therapy for various cardiovascular diseases, such as angina, hypertension, hyperlipidemia [7].

Materials and methods

1) Hawthorn fruit was collected from Normafa, Hungary.
2) Ethanol (≥99 %, REANAL LABOR, Co., Hungary) was used for extraction purpose.
3) The applied chemicals and reagents for the analysis were collected per following list: methanol (LACH-NER, Co., Czech Republic), gallic acid (SIGMA-ALDRICH, Co., USA, product of China), acetic acid (96 %, REANAL LABOR, Co., Hungary), L-ascorbic acid (reagent grade, SIGMA-ALDRICH, Co., USA, product of China), hydrochloric acid (37 %, CARLO ERBA Reagents S.A.S, France), Folin-Ciocalteu reagent (SIGMA-ALDRICH, Co., USA), sodium acetate (Analar grade, VWR Chemicals, Belgium). 2,4,6-Tris(2-pyridyl)-s-triazine (≥99 %, SIGMA-ALDRICH, Co., USA), sodium carbonate (MERCK KGaA, Germany), ferric chloride (REANAL LABOR, Co., Hungary).

Raw materials and extraction methods:

After removing the sticks and the crushed fruit, Hawthorn fruit was washed and cleaned, and dried from the water, then Shredding of the different portions was done using GM 200 pulverizer, the (UAE) process was performed by using (ULC PREMIUM,WEBER ULTRASONICS AG, Germany) with power 100 W this apparatus available in livestock and food preservation technology department, during (3, 6, 9 minutes), and by using (0.05. 0.075, 0.1 g/ml sample-solvent ratio) by using 10% ethanol and distilled water.

Total phenolic compounds (Folin-Ciocalteu method):

Total phenolic compounds (TPC) were estimated by the Folin-Ciocalteu method [8]. The absorbance was measured at 760 nm Gallic acid (0.3 mM) was used as the standard and the amount of TPC was calculated as: $TPC = (A \cdot 2500 \cdot DF)/(S \cdot a)$ [(mg GAE)/L]

Antioxidant activity (AA) assays (FRAP Method):

The ferric reduction antioxidant power (FRAP) method was applied to quantify antioxidant activity. Reading the absorbance at 593 nm was performed after exactly 5 minutes of incubation. The calibration was realized with ascorbic acid (10 mM). The calculation was done using the following equation: $AA = (A \cdot 1550 \cdot DF)/(S \cdot a)$ [(mg ASE)/L]

Where A is the absorbance; S is the amount of sample (µL); α is the slope of the calibration curve; DF is the dilution factor.

Statistical analysis:

All experiments were repeated twice. Two-way analysis of variance and Tukey's test with 95% confidence level were employed to compare the significant differences among results using software SPSS (version 27.0).

Results and discussion

Total phenolic compounds:

As shown in Figure 1, samples extracted by using 10 % ethanol at (9 min, 0.05 g/ml sample-solvent ratio) gave the highest total phenolic content (TPC) with the mean yield (451.106 ± 0.79 mg/L), and lowest extraction efficiency was at (3 min, 0.05 g/ml sample-solvent ratio) with the mean yield (80.453 ± 1,99 mg/L) while the other samples converged, and their average ranged between (132.678 - 273.261 mg/L).

Total phenolic content (TPC) was lower when the distilled water was used as shown in figure 2, the highest yield was obtained at ((3 min, 0.075 g/ml sample-solvent ratio)with the mean (365.289 ± 15.96 mg/L).

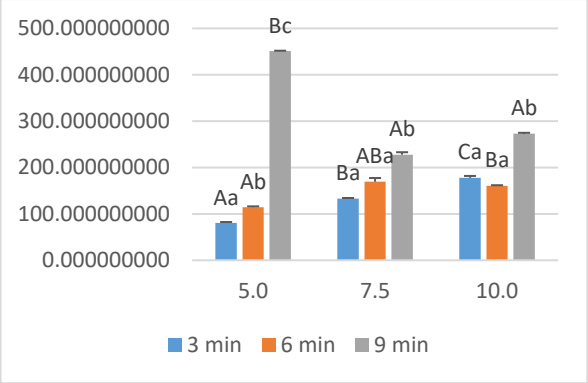

Fig 1: Effects of extraction time and sample ratio on the yield of TPC using 10% ethanol.

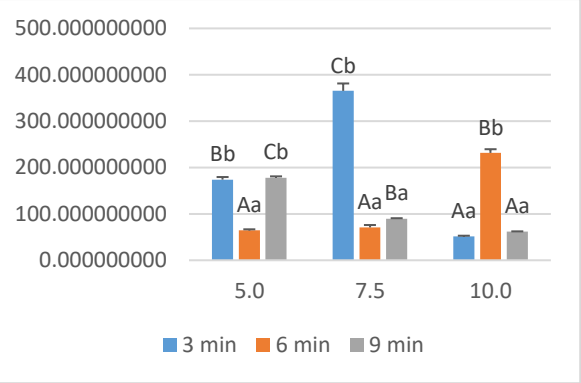

Fig 2: Effects of extraction time and sample ratio on the yield of TPC using distilled water.

Antioxidant activities (AA) assays:

The results of the FRAP analysis (figure 3) showed that the highest antioxidant activity by using 10% ethanol was at (9 min, 0.075 g/ml sample-solvent ratio) , with mean (223.033 ± 1.01 mg/L) while the lowest activity was at (3 min, 0.05 g/ml sample-solvent ratio) with mean (66.9334 ± 0.67 mg/L). It can be seen that the activity of the antioxidants increased with the increase in the extraction time, regardless of the percentage of the sample.

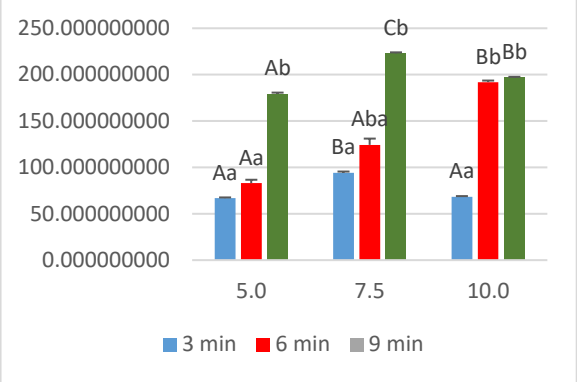

Fig 3: Effects of extraction time and sample ratio antioxidant activity using 10% ethanol.

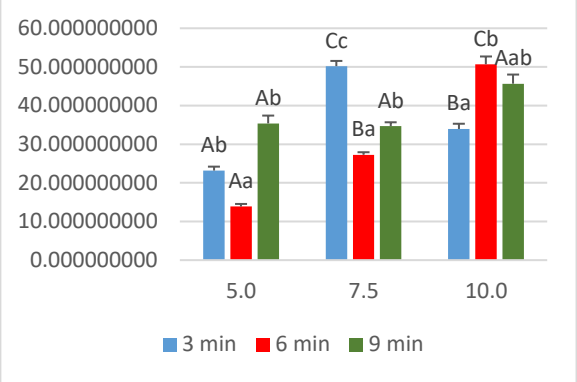

Fig 4: Effects of extraction time and sample ratio antioxidant activity using distilled water.

The figure 4 showed that the highest antioxidant activity by distilled water was at (6 min, 0.1 g/ml sample-solvent ratio), with mean (50.678 ± 2.02 mg/L) as well as at (3 min, 0.075 g/ml sample-solvent ratio) the antioxidant activity was very close with mean (50.2 ± 1.35 mg/L), while the lowest activity was at (6 min, 0.05 g/ml sample-solvent ratio)with mean (13.864 ± 0.67 mg/L).

It can be seen that extraction time has a clear effect on the total content of phenols and the activity of antioxidants. It is expected that it increases with the increase in the extraction time, but the extraction time is a critical parameter in the extraction process, and it has a complex effect. Where during the extraction process, the solvent needs time to diffuse into the cell, and then the analytes react with the solute and dissolve into the extraction solvent, finally, the target compounds diffuse out of the matrix and transfer into the liquid phase. Therefore, the extraction time depends on the time required for the dissolution and diffusion process. The possible reason might be that inadequate extraction time would cause incomplete neutralization for the analytes and insufficient dissolution and diffusion. However, too long an extraction time would lead to structural destruction and decomposition of target analytes [9]. Also, the effect of the sample ratio and its surface area can be explained by this multi-stage process for the diffusion of the solvent and its ability to extract.

Different techniques and solvents, such as ultrasound, microwave, supercritical fluid, leaching, and heating under reflux, have been introduced to extract bioactive substances from the Hawthorn plant. [10] concluded that deionized water extract and 80% acetone extract showed better antioxidant activities compared to ethanol 80% and methanol 80% and that deionized water extract showed a significantly different FRAP from other three extracts during using the UAE, while [11] indicated milk and whey were better than distilled water in (Tannins, Pectic substances, Food fibers) extraction process at different temperatures.

Conclusion

The effect of three times (3, 6, 9 minutes) and three different percentages for the sample (0.05, 0.075, 0.1 g/ml sample-solvent ratio) were studied, and the results indicated a significant effect of each on the extraction process. By measuring the total content of phenols, it was found that the best content (451.106 ± 0.79 mg/L) and (365.289 ± 15.96 mg/L) were obtained at (9 min, 0.05 g/ml sample-solvent ratio), (3 min, 0.075 g/ml sample-solvent ratio) using 10% ethanol and distilled water, respectively. The extracts gave the highest oxidation activity at (9 min, 0.075 g/ml sample-solvent ratio) with a mean (223.033 ± 1.01 mg/L) by using 10% ethanol, while the best value was (50.678 ± 2.02 mg/L) at (6 min, 0.1 g/ml sample-solvent ratio) using distilled water. according to the results, it can be said that it is possible to use distilled water as a solvent to extract the active substances, but it needs further study and the use of other conditions to reach better conditions for the extraction process.

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