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Dedicated to Professor Valer Fărcăşan at his 85th anniversary

EXTRACTION EFFICIENCY OF FLAVONOIDS FROM V*ACCINIUM MYRTILLUS* L LEAVES USING DIFFERENT EXTRACTION TECHNIQUES AND SPECTORPHOTOMETRIC QUANTIFICATION

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ABSTRACT. This paper studies the extraction efficiency of some flavonoids from Vaccinium Myrtillus leaves using different methods: Soxhlet extraction, microwave extraction and sonication.

Each extraction technique was optimized according to characteristic parameters (extraction time, extraction solvent volume and composition of extraction system).

The total flavonoid content was measured spectrophotometrically, as indicated in Romanian Pharmacopoeia (RF X). The obtained results were compared.

Key words: *Vaccinium myrtillus L. Leaves, Soxhlet extraction, Microwave extraction, Sonication, Flavonoids.*

I. INTRODUCTION

Vaccinium Myrtillus L. (blueberry) belongs to the genus *Vaccinium*, widely spread over all the world, with over 200 species of evergreen and deciduous woody plants varying from dwarf shrubs to trees. The genus *Vaccinium* includes many economically importantfarmed small fruit species, like blueberries and cranberries. It grows in Europe and Asia, especially in Scandinavia, Eastern Europe and at high altitides in Southern Europe.

Blueberries, rich in anthocyanins, have been found to be beneficial for health [1, 2, 3]. The flavonoids contained in leaves have diuretic and antibacterial actions [4, 5]. Fruits and leaves were used in traditional medicine in Europe as early as the Middle Ages [6].

Phytotherapy is often using finaly ground medicinal and aromatic plants, where all the compounds occuring in the plant are present, and also raw extracts containing only substances soluble in the extraction solvent used [7]. Different methods can be employed to identify and quantify the bioactive compound or to obtain the raw extract: traditional methods (Soxhlet extraction, solvent reflux) and modern methods (sonication, microwave assisted extraction, superfluid extraction, presurized fluid extraction) [8, 9].

The total content of compound belonging to a specific class, in this case flavonoides can be determined using spectrophotometric methods [10].

II.EXPERIMENTAL

Plant material

Vaccinium Mirtyllus follium from Fares Bio Vital (Bucharest) was finaly ground and then Soxhlet extracted with chloroform to remove chlorophyll and other lipophilic compounds.

Extraction procedure

Flavonoids from 1g samples were extracted using different techniques: reflux extraction, Soxhlet extraction, ultrasound solvent extraction (sonication) and microwave extraction.

A) Reflux extraction was done using 50ml methanol for 30min. After filtration the methanolic extract was evaporated at 75^0C and then dissolved in 10ml methanol. The obtained extract was named S_1. The extract named S_2 was obtained in similar conditions but using a mixture of methanol-water (70:30, v/v) as extraction agent, instead of methanol.
B) Soxhlet extraction was done for 5h using 100ml methanol. The methanolic extract was concentrated at 75^0C and the dry residuum was dissolved in 10ml methanol. The obtained extract is S_3.
C) The ultrasound extraction was performed in a ultrasonic bath (35kHz) and optimized for:
   - extraction time,
   - solvent volume,
   - solvent composition.

The extraction conditions are shown in table 1.

Table 1.

The experimental conditions for ultrasound extraction.

Sample	Time (min.)	Volume (ml)	Solvent composition
S_4	15	30	MeOH
S_5	30		
S_6	45		
S_7	60		
S_8	30	10	MeOH
S_9		20	
S_5		30	
S_{10}		40	
S_{11}		50	
S_{12}	30	50	MeOH - H_2O (90:10, v/v)
S_{13}			MeOH - H_2O (70:30, v/v)
S_{14}			MeOH - H_2O (50:50, v/v)

D) Microwave assisted solvent extraction was perform at 2,45 GHz under different conditions as shown in table 2.

Table 2.

Microwave assisted solvent extraction conditions.

Sample	Volume (ml)	Time (min.)	Power (W)	Solvent composition
The influence of extraction time				
S_{15}	30	1	72	MeOH
S_{16}		2		
S_{17}		3		
S_{18}		3 (each minute 1 min cooling)		
S_{19}		2 (each minute 1 min cooling)		
The influence of microwave power				
S_{20}	30	1,25	96	MeOH
S_{16}		2	72	
S_{21}		1,25	108	
S_{22}		2	81	
The influence of extraction volume				
S_{23}	10	2	72	MeOH
S_{16}	20			
S_{24}	30			
S_{25}	40			
S_{26}	50			
The influence of solvent composition				
S_{26}	50	2	72	MeOH
S_{27}				MeOH - H_2O (90:10, v/v)
S_{28}				MeOH - H_2O (80:20, v/v)
S_{39}				MeOH - H_2O (70:30, v/v)
S_{30}				MeOH - H_2O (60:40, v/v)
S_{31}				MeOH - H_2O (50:50, v/v)
S_{32}				MeOH - H_2O (40:60, v/v)
S_{33}				MeOH - H_2O (30:70, v/v)
S_{34}				MeOH - H_2O (20:80, v/v)

After extraction the samples were filtrated, evaporated to dryness at 75^0C, and then dissolved in 10ml methanol.

Quantitative determination

The total content of flavonoids was determined following the official spectrophotometric method from Romanian Pharmacopoeia (RF X)[10], using $AlCl_3$ as colorimetric reagent. 3ml aluminum chloride (25g/L) was added to 2ml methanolic extract obtained as described above, 5ml sodium acetate (100g/L) and all was brought up to 25ml in a volumetric flask with distilled water. The absorbance was measured after 15min in a 1cm cell, at 430nm. The reference sample was obtained as described above but without adding aluminum chloride. The calibration curve was plotted for rutin (metanolic solution - 0.1mg/ml) as reference compound.

III. RESULTS AND DISCUSSION

The calibration curve and the equation are presented in figure 1. The absorbance for extracts S_1 - S_{34} and the flavonoids concentrationin V*accinium Myrtillus* L. leaves depending upon the extraction condition, are presented in the table 3.

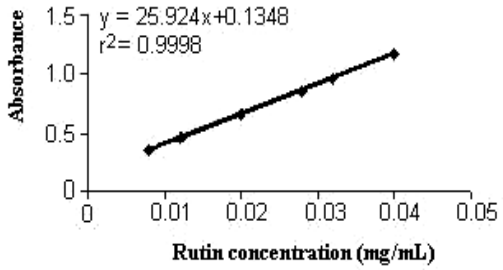

Figure 1. The calibration curve for rutin.

Table 3.

The total concentration of flavonoids compound determined by different extracting techniques.

Sample	Absorbance	Concentration (mg/ml)	Concenration (µg /g*)
Reflux extraction			
S_1	0,5617	4.39 x10^{-3}	164.7
S_2	0,8330	0.0267	267
Soxhlet extraction			
S_3	0,6090	0.0171	171
Sonication (influence of extraction time)			
S_4	0,3548	8.486 x10^{-3}	84.9
S_5	0,3575	8.59 x10^{-3}	85.9
S_6	0,3495	8.282 x10^{-3}	82.8
S_7	0,3512	8.347 x10^{-3}	83.5
Sonication (influence of solvent volume)			
S_8	0,3130	6.874 x10^{-3}	68.7
S_9	0,3500	8.3 x10^{-3}	83
S_{10}	0,4830	0.0134	134
S_{11}	0,5520	0.0161	161
Sonication (influence of solvent composition)			
S_{12}	0,5314	0.0153	153
S_{13}	0,5918	0.0176	176
S_{14}	0,6131	0.0185	185
MASE (influence of extraction time)			
S_{15}	0,2286	3.618 x10^{-3}	36.2
S_{16}	0,2723	5,304 x10^{-3}	53.0
S_{17}	0,2885	5.929 x10^{-3}	59.3
S_{18}	0,3078	9.381 x10^{-3}	93.8
S_{19}	0,3184	7.082 x10^{-3}	70.8

Sample	Absorbance	Concentration (mg/ml)	Concenration (µg /g*)
MASE (influence of power)			
S_{20}	0,2908	6.018 x10^{-3}	60.2
S_{21}	0,3212	7.19 x10^{-3}	71.9
S_{22}	0,3990	0.0102	102
MASE (influence of solvent volume)			
S_{23}	0,2492	4.4128 x10^{-3}	44.13
S_{24}	0,3303	7.541 x10^{-3}	75.4
S_{25}	0,4020	0.0103	103
S_{26}	0,3210	7.183 x10^{-3}	71.8
MASE (influence of solvent composition)			
S_{27}	0,3738	9.22 x10^{-3}	92.2
S_{28}	0,3870	9.728 x10^{-3}	97.3
S_{29}	0,4950	0.0139	139
S_{30}	0,5234	0.015	150
S_{31}	0,6730	0.02	200
S_{32}	0,7650	0.0243	243
S_{33}	0,7637	0.02425	245.5
S_{34}	0,7061	0.022	220

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IV. CONCLUSIONS

Reflux and Soxhlet extraction with methanol as the extraction solvent show good efficiency (S_1 and S_3). When water is added the extraction process improves (S_2).

When sonication is used the total flavonoid concentration decreases. In this case the parameter that has a greater influence is the solvent composition (S_{14}).

Microwave extraction is a modern and efficient technique that shows very good extraction efficiency. The solvent composition has a great influence on the extraction process (S_{32}, S_{33}). The rest of the parameters (volume, time and power) have to be optimized in respect to the solvent composition.

The methods providing the best extraction efficiency are:

- Reflux extraction with a solvent composition: MeOH-H_2O (70:30, v/v)
- Microwave extraction using a solvent composition: MeOH-H_2O (30:70, v/v).

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