

Polyphenol content and antioxidant potential of rhubarb (*Rheum rhabarbarum*) stem by-products depending on solvent polarity and extraction technique

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Abstract: - The aim of the study is to evaluate the effect of solvent polarity and extraction technique on the recovery of antioxidants from rhubarb (*Rheum rhabarbarum*) by-products with single-step – maceration, and ultrasound assisted extraction, and two-step extraction methods - Soxhlet extraction with ethyl acetate, followed by repeated extraction of the residues. Extracts obtained using ethanol showed the highest extract yield, the highest total phenolic content ($78.6 \pm 2.4 \mu\text{g mL}^{-1}$), and exhibited high antiradical scavenging activity (DPPH method), the total flavonoid content (AlCl_3 method) was up to $4.8 \pm 0.2 \mu\text{g mL}^{-1}$. NMR analyses showed that the spectra of non-polar extracts showed the presence of various aliphatic compounds. Findings suggest a sustainable use of rhubarb residues, contributing to waste reduction while exploiting their bioactive potential in cosmetics, pharmaceuticals, and food products due to their strong antioxidant activity.

Key-Words: antioxidants, extraction, solvents, polarity, phenolic, rhubarb, ultrasound

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1 Introduction

The fruit and berry juice processing industry is an important part of global agriculture and food production; however, the residues generated therein still cause significant environmental pollution problems and economic losses, [1], [2]. Fruit processing residues retain a significant content of biologically active compounds, which show high antioxidant activity, [3]. Rhubarb juice production by cold pressing of the rhubarb stalks generates pomace as a residual material or by-product still rich in polyphenolic compounds, such as anthraquinones, stilbenes, flavonoids, and tannins, [4]. Increasingly, scientists are paying attention to the use of fruit by-products as alternative sources of phytochemicals with potential protective effects against cardiovascular disease, cancer, and diabetes, but also for economic reasons, [5].

Although fruit by-products are a cheap, sustainable, and economically promising and untapped resource, their effective use is limited by the availability of methods that can maximize the recovery of bioactive compounds while minimizing both hazardous chemicals and resource use, [6]. Conventional extraction methods typically use various organic solvents, as well as elevated temperatures, which can affect the activity and stability of thermolabile bioactive compounds. Therefore, it is important to

apply extraction methods that not only increase the yield efficiency but also preserve the functional properties of the extracts, [6].

2 Problem Formulation

Residues from fruit and berry juice production, often considered waste, are underestimated, despite the fact that they contain significant amounts of biologically active compounds: phenolic acids, flavonoids, and other antioxidant molecules. These antioxidants contribute to cell protection by reducing oxidative stress caused by excessive amounts of reactive oxygen species.

Prolonged oxidative imbalance is associated with the onset and progression of degenerative disorders, including cancer, cardiovascular disease, and neurodegenerative conditions, [7], [8], [9]. The efficiency of phytochemical recovery and the resulting compound profile are governed by multiple parameters, including solvent characteristics, extraction technique, particle size, temperature, and extraction duration. Traditional solid-liquid extraction methods primarily depend on solvent solubilisation properties and the application of thermal or mechanical energy. However, conventional methods often suffer from low yields, long extraction times, and environmental

implications, highlighting the urgent need for innovative alternatives, [10].

2.1 Rhubarb as a Valuable Source of Bioactive Compounds

Rhubarb (*Rheum rhabarbarum*) stands out as a prolific source of bioactive compounds, particularly during its peak growing season in early spring when it is harvested for juice production, [3]. The juice extraction process, typically achieved through cold pressing, leaves behind pomace, a by-product abundant in phenolic compounds such as anthraquinones, stilbenes, flavonoids, tannins, [10]. When evaluating the effects and prevention of polyphenols and antioxidants against certain diseases, such as cardiovascular diseases, cancer, and diabetes, their potential recovery from fruit and berry by-products is considered very high, [11].

Oxidative stress, caused by an imbalance between reactive oxygen species production and antioxidant defenses, is associated with many diseases, including cardiovascular disease, neurodegenerative disorders, and forms of cancer, [12]. Antioxidants neutralize free radicals and thus protect cells from damage, [13], [14]. Sources of antioxidants include fruits, berries, vegetables, herbs, and other plant materials, which are increasingly used as ingredients in functional foods and dietary supplements, [15].

2.2 Need for innovative methods for extracting biological compounds

Although fruit by-products, a reservoir of bioactive compounds, are sustainable and have low costs, their practical use is highly dependent on the technologies used and the extraction conditions. There are several factors by which the nature of extracted compounds, like phenolic compounds of different polarities and chemical characteristics, varies, [16].

Despite the widespread use of traditional extraction methods, with lower yields, long extraction times, and environmental impacts, these problems create a need for more efficient and alternative extraction methods. Innovative extraction technologies may include: so-called "Green" solvent extraction, using non-toxic, biodegradable solvents that would reduce environmental impacts while maintaining extraction efficiency, [17], [18]. Ultrasound frequency, intensity, temperature, and time can directly affect both extraction efficiency and yields. In addition, types of solvent, solvent volume, as well as sample characteristics such as the moisture content of the sample and particle size are also important factors for effective extraction, [19].

2.3 Environmental and Economic Considerations

Disposal of fruit-processing residues imposes substantial environmental pressure while simultaneously representing a loss of potentially valuable raw materials within the agro-food sector, [1], [9]. Utilization of fruit waste for phytochemical recovery not only reduces waste but also offers new sources of biomolecules that may provide protective benefits against chronic diseases like cancer and diabetes, [20].

3 Materials and Methods

Rhubarb (*Rheum rhabarbarum*) by-products (RBP) for the research were kindly provided by local juice and cider producers Abavas winery, "Kalējkrāmi", Ārlavciems, Slampes municipality, Tukums, Latvia. The rhubarb was harvested and processed during June-July 2025. Rhubarb stalk by-products were cut, excess moisture pressed, and conventionally dried ($45\pm 2^{\circ}\text{C}$, 24 h) with the Rapid Food Dryer DHS600, STOLLAR.

Moisture content of dried RBP: 10-11%. After grinding with the Sample Mill, and kept in the dark at room temperature ($20\pm 2^{\circ}\text{C}$) in plastic boxes for further extractions.

All of the solutions of chemicals were freshly prepared prior to use, and all reagents were commercially available.

3.1 Extraction

Rhubarb (*Rheum rhabarbarum*) by-product extractions are presented in Figure 1 (Appendix). All extractions were performed in a ratio of $1\pm 0.01\text{g}$ dry RBP or extraction residue (ER) to 100 mL of solvent according to methods A to D (1:100 w/v). Each extraction was performed in duplicate ($n = 2$), and Analytical measurements for each sample were performed using three replicates ($n = 3$). Until further analysis, the extracts were stored in a refrigerator at a temperature of $+4\pm 0.1^{\circ}\text{C}$, in closed glass containers.

3.1.1 Single-step extraction

Method A - maceration at room temperature. The dried and ground RBP was transferred to a glass container, and extractions were performed with solvents of different polarity: distilled water, ethanol, methanol, acetone, cyclohexane, isopropanol, ethyl acetate, dichloromethane, acetonitrile, toluene, methyl tert-butyl ether. The vessel was closed and shaken over a 24 - hour period by a continuous orbital

shaker (120 rpm⁻¹). After it was filtered through pleated filter paper and analyzed.

Method B - extraction of RBP with 40% ethanol (EtOH) at varying temperatures (40°C, 60°C, 80°C) over different time intervals (1, 2, and 3 hours).

Method C: Ultrasound-assisted extraction (US) was performed in an ultrasonic processor UP200S Hielscher Ultrasound Technology, Germany, for RBP, [21]. The extraction was performed by continuous ultrasound waves at temperatures of 20°C, 40°C, 60°C, and a frequency of 40 kHz. Solvent used for US extraction - 40% EtOH.

3.1.2 Two-step extraction

Method D - Soxhlet technique with ethyl acetate (EtOAc) was applied for RBP, time for extraction – 2 hours.

After method D, the solid extraction residue (ER) was collected and subjected to a subsequent extraction process with method B, using 40% EtOH at different conditions (20°C (24 hours); 40°C; 60°C; 80°C for 1 to 3 hours)) to maximize the recovery of bioactive compounds that may not have been fully extracted during the initial Soxhlet phase, and to enhance the solubilisation of polar and semi-polar compounds from the treated material, leveraging the solvent's ability to disrupt cellular matrices and improve mass transfer.

3.2 The Yield of Extract's Dry Matter (DM)

Moisture content determination: solvents from extracts were separated by evaporation using HEIDOLPH 036041021 Rotary Evaporator. Moisture content of solvent-free extracts was determined according to ISO 712:2009 Reference method, and the moisture in RBP was analysed by moisture analyser Kern DBS 60-3 (Germany). The yield of the extracts' dry matter (DM) was calculated (Formula 1).

$$EY = \frac{\text{weight of dried extract, g}}{\text{weight of RBP, g}} * 100 \quad (1)$$

3.3 The Total Content of Phenolics was determined according to the modified Folin-Ciocalteu spectrophotometric method, [22]. To 1.0 mL of RBP extract, 5 mL of Folin- Ciocalteu reagent (diluted 10 times with distilled water) was added. After 3 minutes, 4.0 mL 7.5% sodium carbonate (Na₂CO₃) solution in water was added. The resulting solution was mixed and allowed to stand for 30 minutes at 20±1 °C in a dark place. Absorption was read at 715 nm with the M501 Single Beam Scanning UV/Visible Spectrophotometer, United Kingdom. Results were calculated and expressed as microgram

Gallic acid equivalent per 1 mL of extract (GAE µg mL⁻¹), [23].

Quantification was based on a standard curve (Formula 2):

$$TPC = \frac{(Abs-0.0248)}{10.111} \quad (2)$$

TPC - Total Content of Phenolics (TPC, GAE mg mL⁻¹), Abs - Absorption at λ=715 nm.

3.4 The Total Content of Flavonoids

The total content of flavonoids (TFC) in extracts was determined following the aluminium chloride (AlCl₃) colorimetric method, [24]. To 0.5 mL of RBP extract, 1.5 mL 95% ethanol, 0.1 mL 10 % AlCl₃ solution in methanol, 0.1 mL of 1 M potassium acetate CH₃COOK, and 2.8 mL of distilled water were added. After 30 minutes of incubation in the dark, the absorbance was measured at λ=415 nm.

Quantification was based on a standard curve (Formula 4):

$$TFC = \frac{(Abs-0.0148)}{5.9862} \quad (4)$$

TFC - Total flavonoid content (TFC, mg quercetin mL⁻¹), Abs - Absorption at λ=415 nm. Results were expressed as microgram quercetin equivalent per 1 mL of extract (µg mL⁻¹).

3.5 Antiradical Scavenging Activity

Antiradical scavenging activity (A) of RBP extracts was determined with the DPPH method based on scavenging activities of the stable 2, 2-Diphenyl-1-picrylhydrazyl radical, [25], [26]. 4±0.1 mg DPPH reagent was dissolved in 100 mL of 96% EtOH, and the 2 mL of resulting 200µM DPPH reagent was mixed with 2 mL of sample extracts for six different concentrations of each extract (2, 1, 0.7, 0.5, 0.4, and 0.1 mL). The test was done in a test tube and was incubated at room temperature for 30 min. After incubation, the absorbance was measured at λ=515 nm. DPPH inhibition was expressed as the concentration IC₅₀ that inhibited 50% of free radicals with a starting concentration of 100 µM. Antiradical scavenging activity (A, %) was expressed (Formula 3).

$$A = \frac{(AbsC-AbsS)}{AbsC} * 100 \quad (3)$$

A – Antiradical scavenging activity, AbsC – Absorbance of control sample, AbsS – Absorbance of sample.

Nuclear Magnetic Resonance Spectroscopy (NMR) analysis of the extracts was performed, and the compounds in RBP extracts were characterized by a ^1H NMR model Bruker Avance 500 spectrometer.

A mathematical analysis of the data was performed using MS Excel Data Analysis, ANOVA, Single-Factor, Correlation, and Regression analysis. The protruding hypotheses have been tested with a p-value method, and the factors have been evaluated as relevant if $p < \alpha = 0.05$. In the analysis of variance, the Tukey and Friedman tests were used to justify the differences in the results between the studied samples. Statistically significant differences between results were calculated at the level of confidence $\alpha = 0.05$. To find out if the differences in mean values estimated were statistically significant, the One-way analysis of variance was applied and analysed using Microsoft Excel 2016.

4 Results

4.1 The yield of extracts

Single-step extraction with solvents of different polarities robustly depends upon the variety of solvents used for extraction.

Figure 2 A and Figure 2 B (Appendix) show the yields of RBP extracts conducted by maceration at room temperature for 24h.

The results indicated that single-step extractions with other solvents yielded significantly lower amounts than those using water or ethanol, particularly at higher concentrations.

The highest extraction yield was reached with EtOH 96%, establishing it as the most effective solvent for extracting bioactive compounds in this study. This was followed by EtOH 40% and EtOH 50%, respectively.

Solvents with lower polarity, including acetonitrile (5.3%), isopropanol (8.5%), ethyl acetate (4.3%), dichloromethane (4.16%), and tert-butyl methyl butyl ether (3.3%), yielded the least effective extraction results, highlighting their inefficiency for this application.

Using 40% ethanol (Figure 2 C, Appendix) demonstrated that moderate temperatures (40°C) and an extraction time of 2 hours yielded the best results, 12.8%. Higher temperatures (60°C and 80°C) did not consistently provide significant improvements in the extract's yield, and prolonged extraction time resulted in a decrease in the yield. This proves that

both temperature and extraction time are very important for extractions to be maximally effective, while ensuring that the resulting extracts have high antioxidant activity.

Ultrasound-assisted extraction (UAE) with 40% EtOH resulted in the following yields: $5.19 \pm 0.2\%$ for extraction at 20°C; $5.5 \pm 0.1\%$ and $6.2 \pm 0.2\%$ for extraction at 40°C and 60°C, 10 min, respectively.

The ultrasound-assisted extraction (UAE) of rhubarb by-products demonstrated that higher extraction temperatures can significantly improve yield when using 40% ethanol as a solvent. Overall, the findings suggest that UAE combined with ethanol at elevated temperatures is an effective method for maximizing the extraction of valuable compounds from rhubarb by-products, [27].

Two-step extraction with EtOAc

The first step involved extracting with EtOAc, followed by extracting the remaining dry matter with 40% EtOH at various temperatures (40°C, 60°C, and 80°C).

The yield of extract after Soxhlet extraction method with EtOAc reached $8.62 \pm 0.26\%$ (method D).

The yields of re-extraction of RBP (DM) after Soxhlett observed during this extraction process demonstrated the influence of temperature and time on the effectiveness of compound recovery (8.48 – 11,67%) (Figure 1D, Appendix). The most effective results were observed at 40°C during the first two hours of extraction. Two-step Soxhlet-ethyl acetate-ethanol extraction is an example of how to increase the yields of bioactive compounds. This methodology not only improves extraction efficiency but also ensures sustainable solvent use, adding significant value to the field of natural product extraction and compound recovery.

4.2. Total phenolic content

The total phenolic content (TPC, Table 1 (Appendix)) in extracted samples with solvents of different polarities at room temperature for 24 hours highlights the differences in phenolic concentration. EtOH 40-70% yielded the highest phenolic content, with $78.6 \mu\text{g mL}^{-1}$ at 50%, and $77.7 \mu\text{g mL}^{-1}$. Water – $39.2 \mu\text{g mL}^{-1}$ suggests moderate effectiveness, better than some organic solvents. Methanol as a solvent performed moderately well - $43.6 \mu\text{g mL}^{-1}$, similar to low EtOH concentrations. Low TPC was achieved with less polar solvents: acetone: $16.0 \mu\text{g mL}^{-1}$ and isopropanol: $27.2 \mu\text{g mL}^{-1}$. EtOAc reflects limited phenolic extraction capacity – $3.2 \mu\text{g mL}^{-1}$. Non-polar solvents (dichloromethane, acetonitrile, toluene, tert-butyl methyl butyl ether) show the lowest TPCs, with dichloromethane at $3.1 \mu\text{g mL}^{-1}$ and acetonitrile at $0.1 \mu\text{g mL}^{-1}$, highlighting their

inadequacy for phenolic extraction due to non-polarity. Light polar solvents are generally more effective than highly polar or non-polar solvents. This suggests that a balance of polarity in the solvent enhances phenolic extraction efficiency, making ethanol a most effective choice for phenolic applications.

The correlation analysis between polarity and extract yield revealed a moderate positive relationship, with a correlation coefficient of 0.57. This indicates that as the polarity of the solvent increases, the extract yield also increases. Overall, the analysis shows that moderate polarity is suitable for efficient polyphenol extraction. The moderate correlation between polarity and extraction efficiency further emphasizes the complexity of phenol extraction, where several factors interact to influence the results.

The analysis of TPC in RBP with 40% EtOH (Table 2, (Appendix)) shows that TPC remains rather stable across the duration times from 1 to 3 hours at temperatures from 40°C to 60°C, ranging from 73.3 to 89.2 $\mu\text{g mL}^{-1}$. Raising the extraction temperature to 80°C and prolonged extraction time resulted in TPC from 92.7 to 100.8 $\mu\text{g mL}^{-1}$. This indicates that higher temperatures can enhance phenolic extraction. The subsequent extraction process and the re-extraction of rhubarb residues after EtAOc revealed that the highest phenolic content was at 80°C after 3 hours of extraction (152.5 $\mu\text{g mL}^{-1}$).

The results indicated a statistically significant difference in terms of TPC among extracts with method B and method D, $F=13.83 > F_{\text{crit}}=4.49$, $p\text{ value}=0.002$.

The study shows that the UAE with 40% ethanol is most effective at 60°C ($5.5 \pm 0.6 \mu\text{g mL}^{-1}$), while at room temperature (20°C), moderate extraction ($4.0 \pm 0.1 \mu\text{g mL}^{-1}$) is achieved. Reduced efficiency was observed at 40°C ($3.4 \pm 1.0 \mu\text{g mL}^{-1}$). Increasing the temperature seems to improve the extraction performance, possibly due to increased kinetic energy and solvent penetration.

4.3 Total Content of Flavonoids

Flavonoids are low molecular weight compounds of fifteen carbon atoms arranged in a C6-C3-C6 configuration widely distributed in the plant kingdom, [16]. Quercetin has antioxidant and anti-inflammatory effects that might help reduce swelling, kill cancer cells, control blood sugar, and help prevent heart disease, [24]. In comparing the flavonoid content between the single-step and two-step extraction methods at different temperatures and times, the differences in flavonoid concentration are notable. Single-step extraction results (Table 2 (Appendix)) show that the highest flavonoid content

was observed at 40°C, 1 hour ($3.9 \mu\text{g mL}^{-1}$). Flavonoid content generally decreased with longer extraction times at 40°C and maintained a similar range at 60°C and 80°C. A moderate increase was observed at 80°C, 1 hour ($3.4 \mu\text{g mL}^{-1}$).

Two-Step extraction results (Table 2 (Appendix)) show that ethyl acetate plays a significant role in the extraction of flavonoids from plant materials, [24]. Its moderate polarity (0.228) allows to effectively extract a mixture of polar and non-polar compounds, as it has a relatively low boiling point, which makes it easy to remove from the extract after the extraction process, [28]. This property is advantageous in concentrating flavonoids without extensive heat, which could degrade sensitive compounds, [29]. This makes it a convenient choice for continuous and efficient extraction. The flavonoid content in extracts in the first step – Soxhlet extraction reached $5.3 \pm 0.1 \mu\text{g mL}^{-1}$, (method D). The extraction residue after the Soxhlet TFC was continuously extracted, and the TFC content was found to be $5.9 \pm 0.8 \mu\text{g mL}^{-1}$ at 40°C for 1 hour extraction, significantly surpassing the single-step result under the same condition, $3.9 \pm 0.8 \mu\text{g mL}^{-1}$. Generally, two-step extraction consistently yielded higher flavonoid content compared to single-step, particularly observable at 40°C for 1 hour and across most conditions. The different molecular structures and functional groups of flavonoids can result in different solubility in solvents with different polarities, [30].

4.4. The Antiradical Scavenging Activity

Phenolic compounds, as primary antioxidants, act according to two mechanisms: hydrogen-atom transfer or single-electron transfer, [31]. The antiradical scavenging activity of tested compounds was expressed as a relative or absolute decrease in the concentration of DPPH or as IC_{50} (concentration of a compound decreasing the absorbance of a DPPH solution by 50%), [32]. This reaction rate strongly depends on the solvents; it was established that the reaction is faster in polar solvents than in nonpolar ones. The rate of reaction of various antioxidants with DPPH differs. In DPPH tests, the IC_{50} for samples for single-step extraction with solvents of different polarities showed the decrease of concentration of DPPH (IC_{50} from 3.03 in MeOH extracts to 20.66 in EtOH extracts), and displayed varied efficiency across different concentrations, with IC_{50} values ranging from 11.5 to 28.3 $\text{IC}_{50} \mu\text{g mL}^{-1}$. Other solvents like ethyl acetate, dichloromethane, acetonitrile, toluene, and methyl tert-butyl ether exhibited no capability to scavenge DPPH radicals, suggesting limited utility in extracting phenolic compounds with antiradical scavenging activity from

RBP. Samples extracted with water, methanol, and ethanol showed high antiradical activity, suggesting that these solvents efficiently extract compounds capable of neutralizing free radicals, despite the lower phenolic levels. These findings underline the complex nature of antioxidants and highlight the importance of solvent choice in maximizing the therapeutic and pharmaceutical potential of plant extracts.

4.5. Nuclear magnetic resonance (NMR) spectroscopy

As the extracts obtained with unpolar or polar proton solvents revealed the lower polyphenol content in comparison to the extracts obtained with various alcohols or their mixtures, they were additionally analyzed by ¹H NMR spectra (Figure 3 (Appendix)). The spectra revealed presence of various aliphatic compounds.

The less polar cyclohexane extract contains various unsaturated compounds that are approved by the multiplet at 5.3 ppm (characteristic of vinyl protons), some of which should be referred to as free fatty acid (signal at 2.7 ppm assigned to R-CH₂COO-R).

Although the composition of toluene extract consists of aliphatic compounds, herein the presence of various oxygen-containing compounds is detected – signals from 4 to 4.3 ppm are characteristic for alkoxy moieties. Even more, herein, a signal pattern dd and dd is detected, which indicates the presence of triglycerides. When the polarity of solvents is increased, the presence of alkoxy-substituted moieties is increased. The composition of the acetone extract is strongly different from all the previously described herein intensive aromatic signals (7.5 to 8.0 ppm), indicating the presence of benzene rings. The provided signal pattern is characteristic of an o-substituted system.

5 Conclusion

Rhubarb by-products are a significant resource for polyphenol recovery with appropriate extraction methods and solvents, as well as by using efficient, environmentally friendly methods, e.g., ultrasonic extraction. The obtained extracts have significant potential for various industrial applications in the future, due to their strong antioxidant activity. These findings suggest a sustainable use of rhubarb residues, contributing to waste reduction while exploiting their bioactive potential in versatile commercial cosmetics, pharmaceuticals, and food products.

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Contribution of Individual Authors to the Creation of a Scientific Article (Ghostwriting Policy)

Conceptualization and methodology, D.K. and I.M.; writing—original draft preparation, D.K.; writing—review and editing, D.K. and I.M.; visualization, I.M.; supervision, I.M.

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Conflicts of Interest

The authors declare no conflict of interest.

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APPENDIX

RHUBARB (*Rheum rhabarbarum*) BY-PRODUCTS

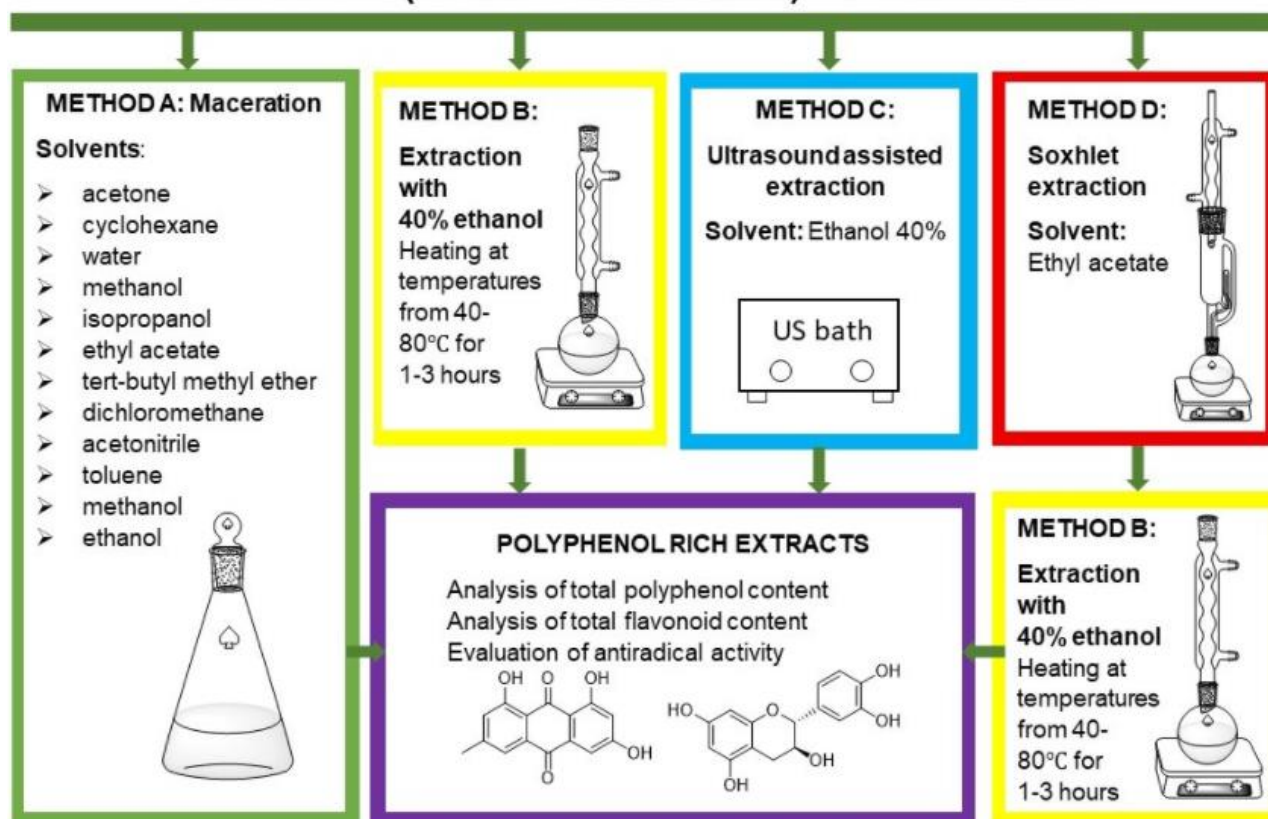


Fig.1: Schematic attachment of the Rhubarb (*Rheum rhabarbarum*) by-product's extraction process.

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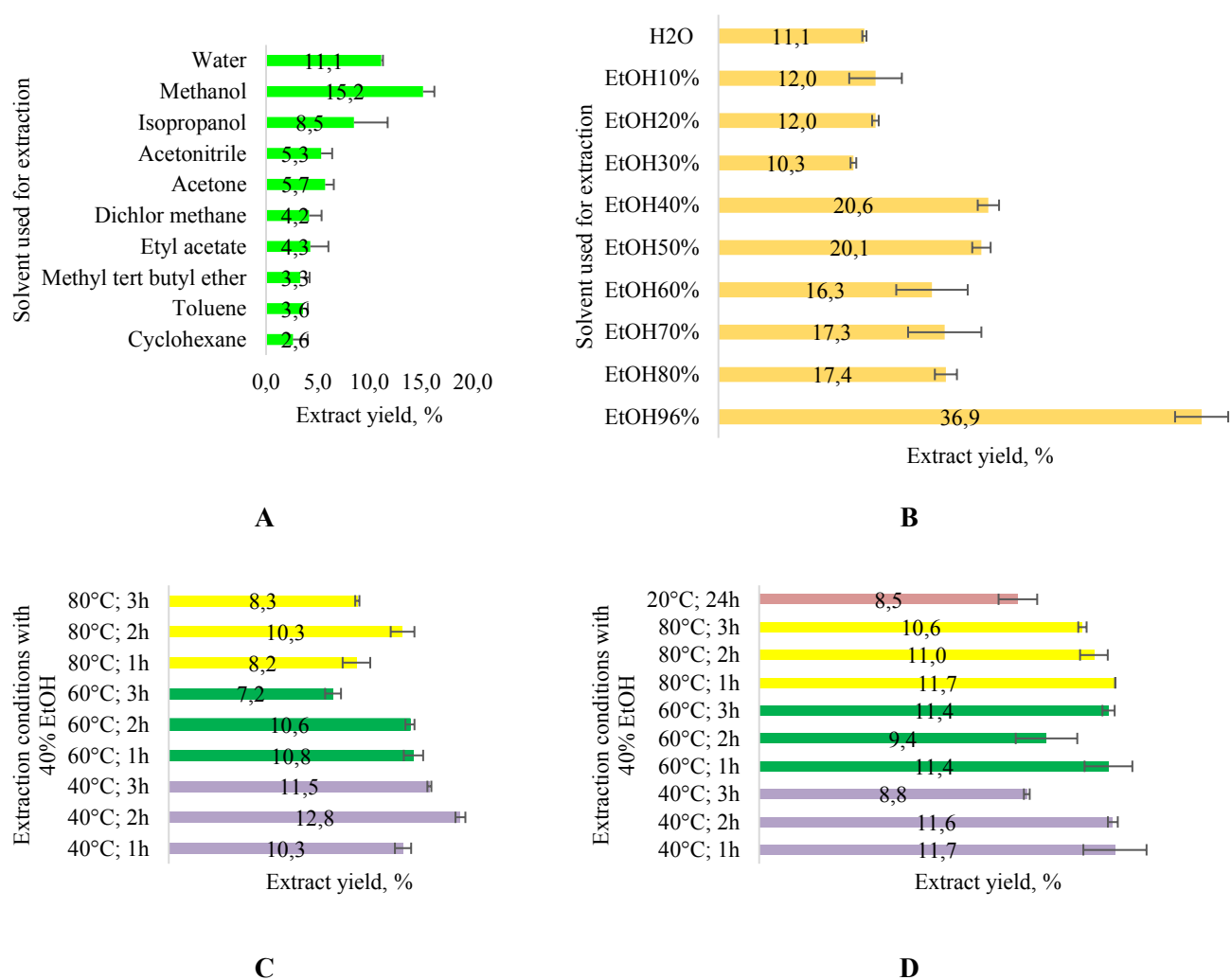


Fig. 2: Extraction yields of RBP extracts

A - Extraction yields of RBP with Single step extraction with solvents of different polarities, method A; **B** - Extraction yields with different concentrations of EtOH at $T=20\pm1^{\circ}\text{C}$, method A; **C** -Single-step Extraction yield of RBP with 40% EtOH, method B; **D** – Re-extraction yield for two-step extracts after EtOAc of extraction residue ((ER) (with 40% EtOH, method B)).

All extractions were done in duplicate ($n=2$), and all measurements were carried out for three independent samples ($n=3$)

Source: created by the authors.

Table 1. Total Content of Phenolics, Total Content of Flavonoids, and Antiradical Scavenging Activity of Rhubarb By-products (*Rheum rhabarbarum*) Extracts

No	Solvent/ extraction conditions	Extraction method	TPC, $\mu\text{g mL}^{-1}$	TFC, $\mu\text{g mL}^{-1}$	IC ₅₀ $\mu\text{g mL}^{-1}$
Single-step extraction by maceration					
1	H ₂ O	A	39.2±0.7	n.d.	21.5±3.7
2	EtOH: H ₂ O (1:9)	A	44.4±0.8	n.d.	15.9±0.2
3	EtOH: H ₂ O (1:4)	A	47.9±1.0	n.d.	11.5±1.2
4	EtOH: H ₂ O (3:7)	A	63.7±1.1	3.0±0.1	20.7±14.6
5	EtOH: H ₂ O (2:3)	A	77.7±0.6	3.0±0.2	20.3±1.6
6	EtOH: H ₂ O (1:1)	A	78.6±2.4	3.6±0.2	13.7±0.8
7	EtOH: H ₂ O (3:2)	A	58.7±5.1	3.6±0.3	16.3±5.6
8	EtOH: H ₂ O (7:3)	A	68.1±1.6	3.8±0.1	20.7±1.3
9	EtOH: H ₂ O (4:1)	A	52.2±2.0	4.2±0.2	11.5±8.6
10	EtOH 96%	A	30.6±1.1	4.8±0.2	28.3±1.2
11	Acetone	A	16.0±0.8	3.1±0.1	n.c.
12	Cyclohexane	A	7.5±1.5	n.d.	n.c.
13	Methanol	A	43.6±0.6	n.d.	30.3±1.5
14	Isopropanol	A	27.2±2.4	n.d.	230.9±24.3
15	Ethyl acetate	A	3.2±0.2	n.d.	n.c.
16	Dichloromethane	A	3.1±0.3	n.d.	n.c.
17	Acetonitrile	A	0.1±0.0	n.d.	n.c.
18	Toluene	A	5.2±0.1	3.3±0.3	n.c.
19	Methyl tert-butyl ether	A	14.5±1.8	3.0±0.1	n.c.
Single-step extraction Ultrasound assisted extraction(UAE), 10 min					
20	EtOH: H ₂ O (2:3); 20°C	C	29.1±0.5	4.0±0.1	14.2±1.5
21	EtOH: H ₂ O (2:3); 40°C	C	32.5±2.3	3.4±1.0	14.0±0.5
22	EtOH: H ₂ O (2:3); 60°C	C	38.9±1.5	5.5±0.6	13.8±0.5
Two-step extraction					
23	Ethyl acetate, Soxhlett;2 h	D	37.2±3.9	5.3±0.1	n.c.

TPC - Total Content of Phenolics; TFC - Total Content of Flavonoids; IC₅₀ - concentration of a compound decreasing the absorbance of a DPPH solution by 50 %. All measurements were carried out for three independent samples (n=3). The test was repeated for five different concentrations (0.1 – 2 mL) of each extract, n.c. - not comparable (the results differ/ are too high (IC₅₀ $\mu\text{g mL}^{-1}$ > 230 IC₅₀ $\mu\text{g mL}^{-1}$)); n.d. – not detected (the results are not detected or TFC < 3.0 $\mu\text{g mL}^{-1}$).

Source: created by the authors.

Table 2. Total Content of Phenolics, Total Content of Flavonoids, and Antiradical Scavenging Activity of Rhubarb By-products (*Rheum rhabarbarum*) Extracts with Single-Step and Two-Step Methods with 40% EtOH

Extraction temperature, time	Single step extracts with 40% EtOH, method A and B			Two-step extracts after EtOAc with 40% EtOH, method E		
	TPC, $\mu\text{g mL}^{-1}$	TFC, $\mu\text{g mL}^{-1}$	IC ₅₀ $\mu\text{g mL}^{-1}$	TPC, $\mu\text{g mL}^{-1}$	TFC, $\mu\text{g mL}^{-1}$	IC ₅₀ $\mu\text{g mL}^{-1}$
40°C; 1h	73.3±3.5	3.9±1.6	15.2±0.1	101.5±20.8	5.9±0.8	17.7±0.4
40°C; 2h	89.6±5.3	3.0±0.0	7.4±1.0	92.4±7.8	3.6±0.1	17.2±1.0
40°C; 3 h	88.6±4.3	2.8±0.1	20.1±2.5	105.2±4.2	3.7±0.1	15.8±4.4
60°C; 1h	88.4±3.9	2.8±0.0	16.1±7.4	109.3±10.0	2.8±0.1	9.6±3.4
60°C; 2h	87.3±3.67	n.d.	26.6±0.8	113.3±4.4	4.1±0.1	16.4±3.3
60°C; 3h	89.2±5.1	2.9±0.2	38.8±3.4	118.1±11.1	4.4±0.4	11.7±10
80°C; 1h	92.7±6.4	3.4±0.2	24.3±0.6	151.3±19.9	3.8±0.1	20.3±2.4
80°C; 2h	96.1±8.6	3.1±0.1	10.1±0.6	164.4±4.2	3.9±0.7	3.1±0.0
80°C; 3h	100.8±11.8	3.6±0.1	4.3±0.1	152.5±1.7	3.9±1.4	13.9±4.8

TPC - Total Content of Phenolics; TFC - Total Content of Flavonoids; IC₅₀ - concentration of a compound decreasing the absorbance of a DPPH solution by 50 %. All measurements were carried out for three independent samples (n=3). The test was repeated for at least five different concentrations (0.1 – 2 mL) of each extract, n.c. - not comparable (the results differ/are too high (IC₅₀ $\mu\text{g mL}^{-1}$ > 230 IC₅₀ $\mu\text{g mL}^{-1}$)); n.d. – not detected (the results are not detected or TFC < 3.0 $\mu\text{g mL}^{-1}$).

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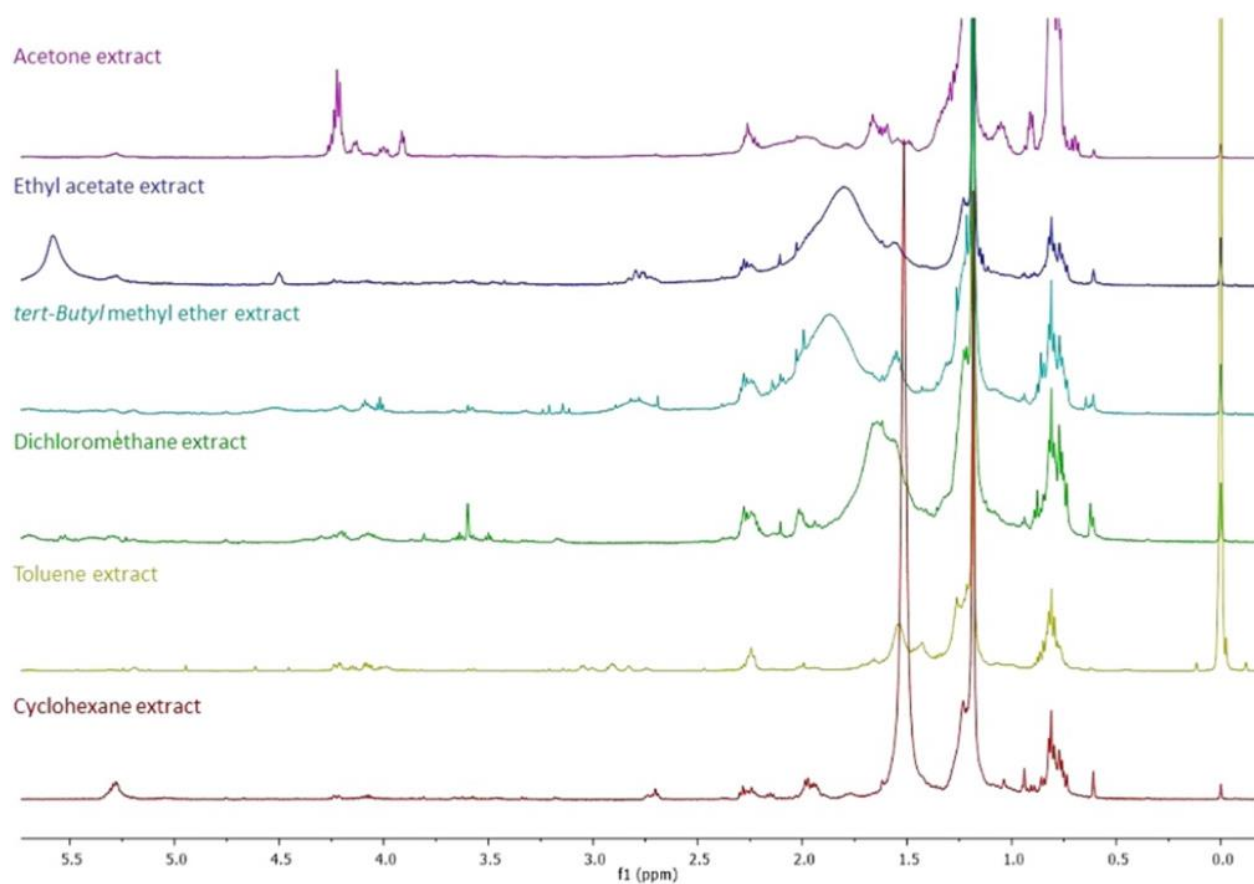


Fig. 3: Nuclear Magnetic Resonance Spectroscopy of RBP extracts with different solvents.
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