# Study of the Ultrasound-assisted Extraction of Polyphenols from Beech (*Fagus sylvatica* L.) Bark

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The aim of this study was to reach the best efficiency of total polyphenols extraction from beech bark. The impacts of solvent concentration, sonication time, and temperature were investigated relative to the yield of extractives from beech (Fagus sylvatica L.) bark using ultrasoundassisted extraction at 40 kHz ultrasonic frequency. All extracts were characterized quantitatively in terms of total content in polyphenols. The extracts obtained in the optimized conditions were also evaluated qualitatively. Beech bark can be a rich raw material for obtaining bioactive polyphenols. An experimental planning method was described that optimized the process and increased the extraction yield. In the experiments, water and ethanol:water solvent solutions were used. The efficiency of the extraction process was determined based on a factorial analysis of variance. The maximum extraction yield of total polyphenols (72.716 mg gallic acid equivalents/g beech bark) was obtained using a processing time of 20 min, an extraction temperature of 65 °C, and an ethanol solvent concentration of 70%. The extracts obtained under the optimum conditions were characterized to determine potential uses of beech bark extractives. The results obtained indicated that ultrasoundassisted extraction was an efficient method for the extraction of natural compounds from beech bark; thus, this method allows for the full utilization of this abundant and low-cost industrial waste.

Keywords: Ultrasound-assisted extraction; Polyphenols; Beech bark; Green extraction; Fagus sylvatica L.

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#### INTRODUCTION

Although beech (*Fagus sylvatica* L.) is a common and widely used material in the wood industry, beech bark is still regarded as a by-product. The bark is separated as a waste product during wood processing and represents 5 to 7% of the total stem volume. Currently, beech bark is only used for combustion (Hofmann *et al.* 2015). Based on literature data (Hoffman *et al.* 2015) and the knowledge that there is a high quantity of polyphenolic compounds with biological activity in the rhytidome, the authors consider beech bark as a potential important raw material.

Phenolic compounds are mainly derived from plants (Baydar *et al.* 2004; Balasundram *et al.* 2006; Castañeda-Ovado *et al.* 2009; Bujor *et al.* 2015). In plant systems, these compounds play an essential role in growth and development, and are

synthesized in response to harmful outside factors (ultraviolet (UV) radiation, pathogens, insects, predators, *etc.*). In recent years, a special interest has arisen in the area of green chemistry concerning the use of new sources of biomass (Ignat *et al.* 2011). The aim is to obtain biologically active compounds that have applications in different fields. The extraction of these compounds from plant materials is an important step in preserving their active properties. The objectives of an optimal method include extraction of the product with a high yield, minimum contamination of the extract, and avoidance of decomposition during extraction.

In the corresponding literature, there are a number of topics that address the extraction and separation of polyphenolic compounds from different manufacturing wastes (Yang and Zhang 2008; Ignat *et al.* 2011; Both *et al.* 2014; Bujor *et al.* 2015; Tanase *et al.* 2015). New extraction techniques can help identify environmentally friendly methods, reduce solvent consumption, shorten the required extraction time, increase the extract yield, and enhance the quality of the extract have been developed, such as ultrasound-assisted extraction (UAE), microwave-assisted extraction, supercritical fluid extraction, and solvent extraction (Ghitescu *et al.* 2015; Hoffman *et al.* 2015). However, all of these methods are in the continual process of optimization depending on the extraction material that is used. Solvent concentration, extraction time, and temperature are important parameters that need to be optimized for maximum recovery of the target compounds.

Ultrasound-assisted extraction is an important method for extracting valuable compounds from lignocellulosic materials (Zhang et al. 2012; Lai et al. 2013). In recent years, several papers have studied the application of ultrasound-based techniques for the extraction of different active compounds (Lianfu and Zelong 2008; Virot et al. 2010; Wang et al. 2010; Jacotet-Navarro et al. 2015; Liao et al. 2015; Nguang et al. 2017). Ultrasound-assisted extraction is influenced by a number of factors, such as the frequency and power of the ultrasounds, duration of ultrasound application, moisture content, particle size, ratio of solid to liquid, type and concentration of solvent, temperature, pressure, and number of extraction cycles (Ghitescu et al. 2015; Liao et al. 2015; Nguang et al. 2017). Ultrasound-assisted extraction is an improved extraction technique with a high efficiency with regards to time and environmental friendliness (Chen et al. 2015; He et al. 2016; Nguang et al. 2017). This method is the cheapest extraction technique and has the fewest instrumental requirements. Compared with classic extraction methods, the use of UAE for phenolic compounds has been reported as a faster, more efficient, and solvent-saving technique (Toma et al. 2001; Hossain et al. 2012; Xu and Pan 2013; Tao et al. 2014: Xu et al. 2016: Chemat et al. 2017).

The use of ultrasounds in the extraction of active compounds from plants causes cavitation and positive mechanical effects (Clodoveo *et al.* 2013; Ghitescu *et al.* 2015). The increase in the extraction yield is attributed to the phenomenon of cavitation produced in the solvent by the passage of ultrasonic waves (Paniwnyk *et al.* 2001). Cavitation is a physical process that increases the hydrophobic character of the extraction medium. It generates macro-turbulence and perturbation in micro-porous particles of the biomass, which accelerates the diffusion process. Cavitation on the product surface causes surface pealing, erosion, and particle breakdown; it also provides exposure of new surfaces, further increasing mass transfer (Vilkhu *et al.* 2008). The ultrasounds have a mechanical effect that accelerates the release of the organic compounds contained within the plant biomass by disrupting the cell walls. The mechanical effect also improves the penetration of solvents into the sample matrix and increases the area of contact between

the solid and liquid phase (Rostagno *et al.* 2003). This mechanical effect coupled with the enhanced mass transfer and disruption of cells by cavitation bubble collapse releases cellular contents into the bulk medium (Paniwnyk *et al.* 2001).

The UAE of polyphenols from beech bark has not yet been studied in detail. Although beech bark can be a rich raw material for obtaining antioxidant compounds, only one study was found in literature dealing with UAE from beech bark (Hoffman *et al.* 2015).

The objectives of this study were: (1) to elucidate the influence of temperature, sonication time and solvent concentration on yields of extracted polyphenols from beech bark, (2) to describe an experimental planning method that optimizes the process, (3) to determine the phenolic profile by HPLC of the optimized extracts. For this investigation, in addition to pure water, different concentrations of ethanol were used as solvents because these mixtures proved to be more efficient than methanol in this process (Hoffman *et al.* 2015).

#### EXPERIMENTAL

#### Materials

Beech bark was provided by a wood processing company (Sardo Lemn, Vatra Dornei, Romania). Prior to extraction, the beech bark was separated, air-dried at room temperature (10.5% humidity), and milled in a Grindomix GM 2000 (Retsch, Bucharest, Romania). The powder obtained was sifted with a sieve to obtain particles with a diameter of 0.5 mm. The biomass was used directly without any pretreatment.

Ethanol, Folin Ciocalteu's phenol reagent, gallic acid, and sodium carbonate were purchased from Sigma-Aldrich (Taufkirchen, Germany).

#### Methods

#### Ultrasound-assisted extraction

The UAE was performed in an Elma Transsonics ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) at 40 kHz ultrasonic frequency. The set-up allowed for the control of the time and temperature. The beech bark (2.5 g) in three replicates, was placed into a volumetric flask (100-mL), which was filled to volume with the ethanol:water solvent. The ethanol concentrations tested were 50% (v/v) and 70% (v/v). The extraction times were 15 min, 30 min, and 45 min, and the temperatures investigated were 50 °C, 60 °C, and 80 °C. After extraction, the filtrate was collected in a volumetric flask and used to determine the total phenolic content.

#### Characterization of the extracts

Determination of the total polyphenolic content in the beech extracts (three replicates and three readings for each sample) was performed spectrophotometrically (UV-vis spectrophotometer with a wavelength range of 190 nm to 1100 nm; Specord 210, Analytykjena, Jena, Germany) using Folin-Ciocalteu's reagent with a protocol that was previously developed by Tanase *et al.* (2013). The calibration curve was made with a gallic acid standard solution and measurements were taken at 765 nm. The total polyphenolic concentration was expressed in mg gallic acid equivalents (GAE)/g.

#### Experimental design

The experimental data did not fulfill the requirements of the general linear model (normal distribution of the dependent variable within groups, homogeneity of variances); thus the align-and-rank data for nonparametric ANOVA procedure was used for data preprocessing (Wobbrock *et al.* 2011).

To determine the most relevant factors that influence the extraction yield (total phenolic content of beech bark), a factorial analysis of variance (ANOVA) was performed on the aligned rank transformed data (R version 3.4.3, The R Foundation for Statistical Computing, Auckland, New Zealand). The three independent variables of the UAE process were temperature (°C), sonication time (min), and solvent concentration (%), where each variable had three levels. A total of 27  $(3^3)$  combinations of factors were obtained and performed. Each experiment had three replicates and three readings for each sample. Thus, a total of 243 samples were used for the study. The "only effects" (no interactions), two-way interactions, and "all effects" (three-way interaction) of the studied independent variables were analyzed (Ghitescu et al. 2015). The Tukey-corrected test performed the post-hoc comparisons of means. The average yields and standard deviations were also calculated. The data analyses were performed in R statistics (Ismeans library, car and ARTool packages, version 3.4.3, The R Foundation for Statistical Computing, Auckland, New Zealand). For optimization of the independent variables with a significant influence on the UAE of the phenols from beech bark, threedimensional surface plots were constructed according to the best mathematical polynomial model (Ghitescu et al. 2015). The generation of the mathematical models and response surfaces was performed using Systat TableCurve 3D version 4.0 (Systat Software Inc., San Jose, CA, USA). Finally, according to the generated model, a maximum yield was predicted with the optimum conditions using the Matlab program (MathWorks, release R2016a, MA, Natick, USA).

#### Qualitative analysis of the optimized beech bark extract

For the qualitative analysis, an ultra-high performance liquid chromatography (UHPLC) system was used (Flexar FX – 10, Perkin Elmer, Waltham, USA). All of the solvents were HPLC-grade and the reagents used had the highest available purity. The column used was a Luna C18 (Phenomenex, Torrance, USA) that was 150 mm × 4.6 mm and 3  $\mu$ m particle size. The mobile phase was 0.1% V/V formic acid (A) and acetonitrile (B). The elution gradient program was as follows: 0 min to 0.1 min: 90% A, 10% B; 0.1 min to 20.1 min: 90% to 20% A; 20.1 min to 25.1 min: 20% A; 25.1 min to 26.1 min: 20% to 90% A; and 26.1 min to 30.1 min: 90% A. The mobile phase was delivered at a flow rate of 1 mL/min and the column was maintained at 35 °C. The monitoring wavelengths were 270 nm, 280 nm, 324 nm, and 370 nm. A methanol-water mixture of Sigma-Aldrich reference substances was used that contained gallic acid, eleutheroside B, catechine, epicatechine, vanillic acid, sinapic acid, taxifoline, and quercetin. The autosampler temperature was set at 20 °C.

Regarding the reproducibility of the final chromatograms, three control samples for optimized values (extraction with water - FUS A, and with ethanol:water 70:30 - FUS Et-A) were prepared (each with three readings), and then the TPC for all samples was determined.

#### **RESULTS AND DISCUSSION**

#### Effect of the Extraction Parameters on the Total Phenolic Content

The data concerning the experimental variables and obtained total phenolic contents are presented in Table 1. The results of the factorial ANOVA analysis showing the effects of the independent variables and their interactions on the extraction yield are summarized in Table 2. The only effects analysis showed that variables with the most significant effect on the extraction process were the solvent concentration and temperature. The time had no significant effect on the extraction yield (Table 2).

It is shown that a single solvent might not be effective for the extraction of total polyphenols. The extraction of these bioactive compounds depends largely on the polarity of solvents and phenol compounds. Thus, a combination of alcohol with water is more effective in extracting phenolic compounds than alcohol alone (Boeing *et al.*, 2014). The total phenols yield increased with an increase in ethanol concentration from 0% to 70%. This is probably due to the increased solubility of phenolic compounds in the mixture of ethanol and water. The findings obtained from our study are in good agreement with Ghitescu *et al.* (2015), where the phenolic compounds yield from spruce bark increased when ethanol concentration increased.

The higher temperature accelerated the whole extraction *via* increased solubility and diffusion of phenolics, decreased solvent viscosity, mass transfer, and penetration of solvent especially in the bark and wood tissue (Xu and Godberg 2000; Wang *et al.* 2008). At the same time, heating might soften the bark matrix and weaken the phenol-protein and phenol polysaccharide interactions in the plant materials (Shi *et al.* 2003). Thus, more phenolic compounds would transfer to the solvent. Hoffman *et al.* (2015) showed that water at 120 °C was efficient with the use of microwave-assisted extraction; the resulting solutions had excellent antioxidant properties. Regarding identification of phenolic compounds, they found that using pure water or solutions of alcohols at 120 °C were the most efficient methods. On the other hand, a possible degradation of phenolic compounds caused by hydrolysis, redox reactions, and polymerizations are detrimental to the extraction yield and quantification of bioactive compounds (Abad-Garcia *et al.* 2007). Therefore, a very high extraction temperature may evaporate ethanol from the aqueous ethanol solution and subsequently change the ethanol-to-water ratio.

The two-way interactions analysis revealed that the temperature-time interaction had no significant effect on the extraction yield (Table 2, Fig. 1A). No significant differences were observed between TPC means on different sonication times (Tukey-corrected, Post hoc pairwise comparisons). The temperature-solvent concentration and time-solvent concentration interactions both had significant effects on the extraction yield (Table 2, Figs. 1B and 1C). In the case of time-solvent concentration interaction, no significant differences were observed between TPC means at different sonication times (Tukey-corrected, Post hoc pairwise comparisons).

The influence of these factors (temperature and solvent concentration) could be explained by an improved diffusion in the mass transfer process, vegetal tissue degradation, and improvement of the solvent penetration at different concentrations (Sališová *et al.* 1997). In the case of spruce bark (Ghitescu *et al.* 2015), only the interaction of time and ethanol concentration had a statistically significant influence on the extraction yield.

The interactions between all three variables had significant effect on the extraction yield (Table 2, Fig. 2). No significant differences were observed between TPC

means at 30 and 45 min of sonication times (Tukey-corrected, Post hoc pairwise comparisons). The minimum total phenolic content was obtained when the solvent was 100% water, while the maximum content (67.5 mg GAE/g beech bark) was obtained with a 70% ethanol solvent concentration (ethanol:water = 70:30), temperature of 60 °C, and extraction time of 15 min (Fig. 2). Ethanol is known to be a good solvent for polyphenol extraction and is safe for human consumption. Previous studies have also reported that the extraction of polyphenols from plant materials is more effective with an ethanol concentration of approximately 70% (Yang and Zhang 2008; Wang *et al.* 2013; Ghitescu *et al.* 2015). The raw data of the total phenolic content are shown in the Appendix (Table A1).

	Extraction	Extraction	Ethanol	Total Phenolic Content		ontent	
	Temperature	Time	Concentration		(mg GAI	E/g beech	bark)
Run	(°C)	(min)	(%v/v)	1	2	3	Mean±SD <sup>a</sup>
1	50	15	0	36.25	38.1	37.2	37.18±0.801
2	50	15	50	49.87	49.71	49.21	49.59±0.299
3	50	15	70	57.98	57.55	57.81	57.78±0.189
4	50	30	0	33.25	33.64	33.83	33.57±0.257
5	50	30	50	55.45	55.47	55.21	55.37±0.124
6	50	30	70	64.21	64.07	64.87	64.38±0.369
7	50	45	0	33.24	33.51	32.89	33.21±0.268
8	50	45	50	57.98	57.65	58.21	57.94±0.242
9	50	45	70	60.54	60.04	59.87	60.14±0.302
10	60	15	0	40.42	41.54	40.64	40.86±0.512
11	60	15	50	61.01	61.34	60.87	61.07±0.206
12	60	15	70	66.98	67.98	67.54	67.50±0.435
13	60	30	0	41.56	41.98	42.12	41.88±0.249
14	60	30	50	61.98	61.87	61.21	61.68±0.360
15	60	30	70	65.85	65.27	66.26	65.79±0.428
16	60	45	0	44.54	45.02	44.69	44.75±0.210
17	60	45	50	64.21	64.78	64.11	64.36±0.310
18	60	45	70	64.99	64.87	64.11	64.65±0.413
19	80	15	0	44.97	44.87	45.32	45.05±0.204
20	80	15	50	56.54	55.87	56.12	56.17±0.295
21	80	15	70	65.11	65.09	65.49	65.23±0.192
22	80	30	0	44.21	44.12	44.84	44.38±0.341
23	80	30	50	54.65	55.87	56.87	55.79±0.962
24	80	30	70	61.65	61.22	62.18	61.68±0.417

Table 1. Experimental Planning (Each Experiment had Three Replicas a	Ind
Three Readings for Each Sample)	

25	80	45	0	41.98	42.42	41.24	41.88±0.515
26	80	45	50	55.21	54.78	54.58	54.85±0.282
27	80	45	70	61.54	61.99	62.84	62.12±0.569

Note: a- mean ± standard deviation (n=9)

**Table 2.** Only Effects, Two-way Interactions, and All Effects of the StudiedIndependent Variables on the Total Phenolic Content Extracted from the BeechBark (Analysis of Variance of Aligned Rank Transformed Data)

Effects	DF <sup>a</sup>	F-test <sup>b</sup>	<sup>c</sup> Value ک
Only effects, no interactions			μ. · · · · · · · · · · · · · · · · · · ·
Temperature	2	14.003	< 0.00001* <sup>d</sup>
Time	2	0.034666	0.96593
Concentration	2	451.61	< 0.00001* <sup>d</sup>
Residuals	240		
Two-way interactions			
Temperature and time interaction			
Temperature	2	14.85983	< 0.00001* <sup>d</sup>
Time	2	0.060432	0.94137
Temperature x time	4	1.300736	0.2705
Residuals	234		
Temperature and concentration interaction			
Temperature	2	321.519	< 0.00001* <sup>d</sup>
Concentration	2	856.883	< 0.00001* <sup>d</sup>
Temperature x concentration	4	36.764	< 0.00001* <sup>d</sup>
Residuals	234		
Time and concentration interaction			
Time	2	0.14075	0.86877
Concentration	2	440.3382	< 0.00001* <sup>d</sup>
Time x concentration	4	2.61269	0.03615* <sup>d</sup>
Residuals	234		
All effects and interactions			
Temp	2	868.331	< 0.00001* <sup>d</sup>
Time	2	33.907	< 0.00001* <sup>d</sup>
Concentration	2	869.51	< 0.00001* <sup>d</sup>
Temperature × time	4	386.263	< 0.00001* <sup>d</sup>
Temperature × concentration	4	858.87	< 0.00001* <sup>d</sup>
Time × concentration	4	339.167	< 0.00001* <sup>d</sup>
Temperature x time x concentration	8	361.395	< 0.00001* <sup>d</sup>
Residuals	216		

Note: a- degrees of freedom; b- F-statistics (Type III tests); c- significance criterion \* d- significant values where  $p \le 0.05$ 



**Fig. 1.** Two-way interactions effect on the total phenolic content extracted from the beech bark: A- temperature-time; B- temperature-solvent concentration; and C- time-solvent concentration





Optimization of the processing parameters for maximizing the yields of phenolics was performed in accordance with the extraction results. The optimum levels of the two physical variables (temperature and time) were determined at a solvent concentration of 70%.



**Fig. 3.** Surface-fit of the mathematical model with the best equation for extraction with 70% ethanol; experimental values (dots) plotted with error bars

The best mathematical model was chosen on the basis of the response surfaces by taking the best location of the experimental points on the graph, simplest characteristic mathematical equation, and best squared regression coefficient ( $R^2$ ). The surface-fit with polynomial equations created a mathematical model corresponding to Eq. 1 with a  $R^2$  of 0.78 and F-test of 2.22,

$$z = a + b/x + c/y + d/x^{2} + e/y^{2} + f/(xy)$$
(1)

where z is the polyphenol concentration (C, mg GAE/g beech bark), x is the extraction temperature (T, °C), and y is the extraction time (t, min).

The surface plotted in Fig. 3 was obtained with Eq. 2:

$$C = -30.27 + 10433.82/T + 698.33/t - 303564.44/T^2 - 3631.5/t^2 - 21275.86/Tt \quad (2)$$

The response surface analysis demonstrated that the relationship between the total phenolic content and extraction parameters was quadratic with a relatively good regression coefficient. The optimum conditions obtained for an ethanol solvent concentration of 70% using the model were an extraction temperature of 65 °C and time of 20 min. Under the optimum conditions and using Matlab, the model predicted a maximum yield of 67.871 mg GAE/g beech bark (Fig. 4).

To compare the predicted results with practical values, a control experiment was performed three times with the previously obtained optimum conditions. For this extract (FUS Et-A), a mean value of 72.716 mg GAE/g beech bark  $\pm$  1.202 mg GAE/g beech bark was obtained, which was slightly higher than the value predicted by the model. In the study by Hoffman *et al.* (2015), the value for the total polyphenol content (at room temperature, 10 min, ethanol:water = 80:20) was 49.9 mg quercetin/g beech bark. The results of this study suggested that, under high temperature conditions (65 °C), the ethanol:water (70:30) mixture was a more efficient solvent.





Because of the pharmaceutical uses of the extractives, the optimum levels of temperature and time were also determined in the case of water as a solvent. The mathematical model corresponded to the following equation ( $R^2 = 0.87$ , F-test = 4.28):

$$z = a + bx + cy + dx^{2} + ey^{2} + f(xy)$$
(3)

The surface presented in Fig. 5 was obtained with Eq. 4:

$$C = -75.50 + 3.45T - 0.12t - 0.02T^{2} + 0.24 \cdot 10^{-2}t^{2} - 0.92 \cdot 10^{-3}Tt$$
(4)





The optimum conditions obtained for a solvent of 100% water using the second model were an extraction temperature of 72 °C and time of 15 min. Under the optimum conditions, the model predicted a maximum yield of 46.415 mg GAE/g beech bark (Fig. 6).



Fig. 6. Maximum yield prediction for extraction with 100% water solvent

For this extract (FUS A), a mean value of 56.28 mg GAE/g beech bark  $\pm$  2.25 mg GAE/g beech bark was obtained. Hoffman *et al.* (2015) obtained a value of 39.4 mg quercetin/g beech bark for the total polyphenol content at room temperature and a time of 20 min.

Green extraction techniques have many advantages. To list a few are: short extraction time; convenient and economical method; sustainable and eco-friendly polyphenol extraction; reproducibility; an alternative to toxic solvent usage (Ameer *et al.* 2017). Thus, results of this study showed that water was an efficient solvent under high temperature conditions and supported the concept of green extraction.

#### Identification of the Phenolic Compounds using HPLC

The FUS A and FUS Et-A extracts obtained under the optimum conditions were characterized to determine the properties and potential uses of these bioactive compounds. The overlaid chromatograms of a mixture of standards at the reference wavelength of 270 nm and the optimized extracts (FUS A and FUS Et-A) are shown in Fig. 7. Identification was performed using the correspondence of the retention time, multiwavelength analysis, and standard addition method. The concentration of the standard mixture (1:1) was 218 µg/mL gallic acid, 87.2 µg/mL eleutheroside B, 82.4 µg/mL catechine, 124.8 µg/mL epicatechine, 160.8 µg/mL vanillic acid, 86.4 µg/mL sinapic acid, 95.2 µg/mL taxifoline, and 143.2 µg/mL quercetin.



**Fig. 7.** Overlaid chromatograms for: A- FUS A, B- FUS Et-A; and C- MIX8STD (mixture of standards at a reference wavelength of 270 nm); Identified compounds: 1- Gallic acid; 2- Eleutheroside B; 3- Catechine; 4- Epicatechine; 5- Vanillic acid; 6- Sinapic acid; 7- Taxifoline; and 8- Quercetin

The compounds identified in the FUS A extract by HPLC were eleutheroside B (syringin), epicatechine, and sinapic acid. Catechine, epicatechine, and taxifolin were identified in the FUS Et-A extract in small amounts. It is known that sinapic acid shows antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-anxiety properties (Nićiforović and Abramović 2014). The results of this study indicated that the FUS A extract contained a significant amount of sinapic acid. As such, the authors recommend the FUS A extraction conditions to obtain this phenolic compound. In contrast, the FUS Et-A extraction conditions may be used to obtain catechin, which is known to have an antibacterial activity (Friedman *et al.* 2006).

#### CONCLUSIONS

- 1. The influence of temperature, time, and solvent concentration on the extract yield from UAE was evaluated. The results showed that solvent concentration and temperature had the most significant effect on the extraction process.
- 2. The analysis of the two-way interactions revealed that the temperature-time interaction had no significant effect on the extraction yield. The temperature-solvent concentration and the time-solvent concentration interactions significantly influenced the total phenolic content of the beech bark extract.
- 3. The interactions between all three variables had a significant effect on the extraction yield. No significant differences were observed between TPC means on 30 min and 45 min of sonication times.
- 4. The model, under the optimum conditions (65 °C, 20 min, and 70% ethanol concentration), predicted a maximum yield of 67.87 mg GAE/g beech bark. An

experimental value of 72.72 mg GAE/g beech bark  $\pm$  1.20 mg GAE/g beech bark was obtained.

- 5. The results of the control experiments suggested that the developed models can be used to improve the extraction yield with an approximate prediction of the optimum values for the temperature and time at given ethanol concentrations. Further research is needed (that introduces other UAE influencing parameters) to generate an optimized model that appropriately explains the extraction process.
- 6. The compounds identified by HPLC in the optimized beech bark extracts (FUS Et-A and FUS A) were catechine, epicatechine, taxifolin, sinapic acid, and quercetin. These results can be further refined with the identification of all polyphenolic compounds and with biological tests in practical applications.

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#### APPENDIX

	Factors			Response
Dup	Temperature	Sonication time	Solvent concentration	трс
Run	(°C)	(min)	(%v/v)	IPC
1	50	15	100	36.25
2	50	15	100	36.27
3	50	15	100	36.23
4	50	15	100	38.1
5	50	15	100	38.09
6	50	15	100	38.11
7	50	15	100	37.2
8	50	15	100	37.2
9	50	15	100	37.19
10	50	15	70	57.98
11	50	15	70	57.98
12	50	15	70	57.99
13	50	15	70	57.55
14	50	15	70	57.53
15	50	15	70	57.57
16	50	15	70	57.81
17	50	15	70	57.81
18	50	15	70	57.81
19	50	15	50	49.87
20	50	15	50	49.87
21	50	15	50	49.88
22	50	15	50	49.71
23	50	15	50	49.72
24	50	15	50	49.7
25	50	15	50	49.21
26	50	15	50	49.22
27	50	15	50	49.2
28	50	30	100	33.25
29	50	30	100	33.24
30	50	30	100	33.25
31	50	30	100	33.64
32	50	30	100	33.66
33	50	30	100	33.63
34	50	30	100	33.83
35	50	30	100	33.82
36	50	30	100	33.83
37	50	30	70	64.21
38	50	30	70	64.21
39	50	30	70	64.22
40	50	30	70	64.07
41	50	30	70	64.08
42	50	30	70	64.06
43	50	30	70	64.87
44	50	30	70	64.88
45	50	30	70	64.86
46	50	30	50	55.45
47	50	30	50	55.45

## **Table A1.** Raw Data of the Total Phenolic Content (TPC) from Beech Bark(Ultrasound Assisted Extraction)

48	50	30	50	55.46
49	50	30	50	55.47
50	50	30	50	55.47
51	50	30	50	55.47
52	50	30	50	55.21
53	50	30	50	55.22
54	50	30	50	55.21
55	50	45	100	33.24
56	50	45	100	33.25
57	50	45	100	33.24
58	50	45	100	33.51
59	50	45	100	33.52
60	50	45	100	33.5
61	50	45	100	32.89
62	50	45	100	32.9
63	50	45	100	32.89
64	50	45	70	60.54
65	50	45	70	60.55
66	50	45	70	60.53
67	50	45	70	60.00
68	50	45	70	60.04
69	50	45	70	60.03
70	50	45	70	59.87
71	50	45	70	59.88
72	50	45	70	59.86
73	50	45	50	57.08
73	50	45	50	57.90
74	50	45	50	57.99
75	50	45	50	57.97
70	50	45	50	57.65
79	50	45	50	57.65
70	50	40	50	59.00
79	50	40	50	50.21
00	50	40	50	50.2
01	50	40	100	30.22
02	60	10	100	40.42
03	60	10	100	40.42
04	60	10	100	40.42
00	60	10	100	41.34
00	60	10	100	41.55
0/	60	10	100	41.52
00	60	10	100	40.64
89	60	15	100	40.66
90	60	15	100	40.62
91	60	15	70	66.98
92	60	15	70	66.99
93	60	15	70	66.96
94	60	15	70	67.98
95	00	15	70	07.97
96	60	15	70	67.98
97	60	15	70	67.54
98	60	15	70	67.55
99	60	15	70	67.55
100	60	15	50	61.01
101	60	15	50	61.01
102	60	15	50	61.02

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103	60	15	50	61.34
104	60	15	50	61.33
105	60	15	50	61.34
106	60	15	50	60.87
107	60	15	50	60.87
108	60	15	50	60.88
109	60	30	100	41.56
110	60	30	100	41.57
111	60	30	100	41.56
112	60	30	100	41.98
113	60	30	100	41.97
114	60	30	100	41.98
115	60	30	100	42.12
116	60	30	100	42.11
117	60	30	100	42.12
118	60	30	70	65.85
119	60	30	70	65.85
120	60	30	70	65.85
121	60	30	70	65.27
122	60	30	70	65.27
123	60	30	70	65.28
124	60	30	70	66.26
125	60	30	70	66.26
126	60	30	70	66 25
127	60	30	50	61.98
128	60	30	50	61.00
129	60	30	50	61.99
130	60	30	50	61.87
131	60	30	50	61.88
132	60	30	50	61.87
133	60	30	50	61 21
134	60	30	50	61.2
135	60	30	50	61.23
136	60	45	100	44 54
137	60	45	100	44 55
138	60	45	100	44.50
139	60	45	100	45.02
140	60	45	100	45.02
140	60	45	100	45.02
142	60	45	100	44 69
143	60	45	100	44 69
140	60	45	100	44.00
145	60	45	70	64 99
146	60	45	70	64.98
140	60	45	70	65.01
148	60	45	70	64.87
140	60	45	70	64.87
150	60	<u>45</u>	70	64.87
151	60	<u>45</u>	70	64 11
152	00	<u> </u>	70	64 12
152	00	45 75	70	6/ 11
153	00	45 75	50	6/ 21
154	60	40	50	6/ 21
155	60	40	50	64.22
150	60	40	50	61 70
107	00	40	50	04.70

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158	60	45	50	64.78
159	60	45	50	64.77
160	60	45	50	64.11
161	60	45	50	64.11
162	60	45	50	64.12
163	80	15	100	44 97
164	80	15	100	44.98
165	80	15	100	44.30
166	80	15	100	44.37
167	80	15	100	44.07
107	80	15	100	44.00
168	80	15	100	44.80
169	80	15	100	45.32
170	80	15	100	45.32
171	80	15	100	45.32
172	80	15	70	65.11
173	80	15	70	65.11
174	80	15	70	65.12
175	80	15	70	65.09
176	80	15	70	65.11
177	80	15	70	65.08
178	80	15	70	65.49
179	80	15	70	65.48
180	80	15	70	65.49
181	80	15	50	56.54
182	80	15	50	56.57
183	80	15	50	56 52
18/	80	15	50	55.87
185	80	15	50	55.85
100	80	15	50	55.00
100	80	15	50	55.69
107	00	10	50	50.12
188	80	15	50	50.12
189	80	15	50	56.12
190	80	30	100	44.21
191	80	30	100	44.22
192	80	30	100	44.2
193	80	30	100	44.12
194	80	30	100	44.11
195	80	30	100	44.12
196	80	30	100	44.84
197	80	30	100	44.85
198	80	30	100	44.83
199	80	30	70	61.65
200	80	30	70	61.65
201	80	30	70	61.64
202	80	30	70	61.22
203	80	30	70	61.21
204	80	30	70	61.23
205	80	30	70	62.18
206	80	30	70	62.10
200	80	30	70	62.17
207	00	30	50	5465
200	00	30	50	54.00
209	00	30	50	54.05
210	80	30	50	54.65
211	80	30	50	55.87
212	80	30	50	55.87

213	80	30	50	55.88
214	80	30	50	56.87
215	80	30	50	56.88
216	80	30	50	56.85
217	80	45	100	41.98
218	80	45	100	41.98
219	80	45	100	41.99
220	80	45	100	42.42
221	80	45	100	42.4
222	80	45	100	42.43
223	80	45	100	41.24
224	80	45	100	41.25
225	80	45	100	41.23
226	80	45	70	61.54
227	80	45	70	61.54
228	80	45	70	61.55
229	80	45	70	61.99
230	80	45	70	61.98
231	80	45	70	62.01
232	80	45	70	62.84
233	80	45	70	62.85
234	80	45	70	62.82
235	80	45	50	55.21
236	80	45	50	55.21
237	80	45	50	55.22
238	80	45	50	54.78
239	80	45	50	54.77
240	80	45	50	54.77
241	80	45	50	54.58
242	80	45	50	54.57
243	80	45	50	54.58

Solvent concentration: 100- Water, 50- Ethanol:water 50:50 v/v, 70- Ethanol:water 70:30 v/v; TPC-total phenolic content (mg GAE/g beech bark)