Microwave-assisted Extraction of Spruce Bark: Statistical Optimization Using Box-Behnken Design

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Closed-system microwave-assisted extraction was applied to extract constituents from spruce bark, using 96.6% ethanol as an extractant. The influence of the time (1 min to 20 min), temperature (60 °C, 80 °C, and 100 °C), and liquid/solid ratio (8 mL/g dry bark to 12 mL/g dry bark) on the yield of extractives was studied. The effects of all three of the factors were explained. The results revealed that the optimum conditions were a liquid/solid ratio of 12.0 mL/g dry bark, extraction temperature of 100 °C, and extraction time of 13.4 min.

Keywords: Extraction; Microwave extraction; Spruce bark; Extractives

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INTRODUCTION

Research in the last few decades has focused extensively on wood processing waste, such as bark, which is an attractive renewable raw material. Valorisation is a key component of economic lignocellulosic biorefineries (Jablonský *et al.* 2015a,b; Šurina *et al.* 2015).

One of the by-products of wood processing is bark. Bark of trees is a perspective source of phytochemicals and raw material for particleboards or biocomposite materials. The supply of bark as a source of chemical raw materials is constantly renewing itself. Tree bark represents an unexploited source of extractives, particularly polyphenols, which have great importance in industrial and pharmacological practice. Bark contains 2 to 6 times more extractives than does stemwood (Routa *et al.* 2017). The amount of lumbered round wood worldwide is estimated at 3,590,000,000 m^3. Therefore, the annual global amount of bark, using a 10% bark ratio (Krogell *et al.* 2012), is approximately 359,000,000 m^3 (Pásztory *et al.* 2016). The total content of extractives usually corresponds to 20 to 40% of the dry weight of bark. Worldwide, spruce wood is an attractive source for industry. The Finnish forest industry uses on average 23 Mm^3 spruce logs per year, producing *ca.* 0.9 to 1.3 Mt/a of dry spruce bark calculated with bulk density of 380 kg/m^3 and 10 to 15% volumetric bark content in logs (Feng *et al.* 2013). Spruce or pine bark is currently burned, landfilled, or unexploited for forestry and wood-processing or pulp-paper industry. A similar situation is the economical utilization of the bark, which represents the worldwide problem of waste. Since the wood industry in

Slovakia constitutes an important economic activity related to the cultivation, logging of softwood, it is desirable to evaluate potentially more attractive and economically viable uses for by-products generated by processing (Soto *et al.* 2001). In the Slovak Republic, spruce is the most important source for wood processing and pulp and paper production. The annual spruce bark yield in Slovak Republic is as much as 500,000 m^3. A possible way to reduce environmental pollution (waste landfilling or burning) is related to the full utilization of the waste potential, considering that this waste contains interesting compounds that can be valorised. Exploitation of barks extractives should be in the future one of the most important sources of valuable substances – materials, bioactive chemical, adhesives, and at least as a source of upgraded biofuels. The choice of a suitable method for obtaining chemicals from the waste biomass depends on the target (Kreps *et al.* 2017). One of the objectives is to obtain the largest possible content of compounds from waste, and on the other side to get biologically active substances. Characterization and identification of biological activity represent a key tool determining the choice of acceptable methods. In recently published work (Jablonsky *et al.* 2017), 237 bioactive compounds were identified in the bark of softwood. These compounds especially polyphenols have different active properties and were characterized by properties from the data of literature and by computation of ADME (Absorption, Distribution, Metabolism, and Excretion) properties (Jablonsky *et al.* 2017). The extracts isolated from softwood barks contain hundreds of natural compounds (Jablonsky *et al.* 2017), some of which have cytotoxic (25 identified substances), antioxidant (26 substances), fungicidal (20 substances), and antibacterial (42 substances) effects. In addition, some of these substances are repellents (9 substances) and antifeedants (2 substances). Others may cause growth inhibition (8 substances), increase the activity of pheromones or act themselves as pheromones (10). The amount and nature of the extracted substances is highly dependent on the isolation method adopted, which can be done in different ways.

An extraction method and solvent should be chosen after considering the sample matrix properties, chemical properties of the analytes, matrix-analyte interactions, speed, efficiency, environmental friendliness, and cost (Co *et al.* 2012). Furthermore, the extract composition can be affected by a number of factors, such as the species, varieties, fertilization, pesticide use, harvest time, and drying. New extraction techniques have been introduced recently to reduce the amount of solvent required, reduce the operation time, improve the analysis precision, and reduce the sample preparation cost (Jablonský *et al.* 2017; Belwal *et al.* 2018). Microwave extraction is one of these new technologies. Closed-system microwave-assisted extraction (MAE) of extractive substances may be affected by several factors, such as the frequency and power of the microwave, time of applying microwave radiation, moisture content, particle size, solid to liquid ratio, type and composition of the solvent, temperature, pressure, and number of extraction cycles (Yang and Zhang 2011; Hadkar *et al.* 2013; Ghitescu *et al*. 2015).

Microwave-assisted extraction has emerged as an efficient method for the extraction of phytonutrients and is an important technique for extracting valuable compounds from different biomasses (Guo *et al.* 2001; Hao *et al.* 2002; Ananth *et al.* 2010; Zhang *et al.* 2011; Sládková *et al.* 2016). The main advantages of MAE over conventional extraction techniques are that it can reduce the extraction time, uses less solvent, has a higher extraction efficiency, better target extract quality, and lower cost of the extraction technique and process (Chen *et al.* 2007; Zhang *et al.* 2011). Nevertheless, irradiation can accelerate the chemical reactions or changes some of the target substances and other extraction conditions (*e.g.*, extraction pressure) of MAE. Another effect of

MAE is that it may reshape or transform the chemical framework of the target secondary metabolites (Zhao *et al.* 2006; Ghani *et al.* 2008; Zhang *et al.* 2011). Microwave extraction of spruce bark was previously reported in the work of Sladkova *et al.* (2016), which evaluated parameters such as particle sizes (0.3; 1.0; 2.5 mm), time (3 to 20 min), and temperature (60; 80; 100 °C) on polyphenol recovery. In spite of a higher yield of a polyphenol in the case of a particle with 0.3 mm, the results presented in mentioned study show that the subsequent processing of the bark is more suitable for a fraction with particle size of 1 mm. Solvent choice for MAE is dictated by the solubility of the extracts of interest, by the microwave absorbing properties of the solvent determined by its dielectric constant, and by the interaction between solvent and plant matrix (Csiktusnadi Kiss *et al.* 2000). Solvents such as methanol, ethanol, and water are sufficiently polar to be heated by microwave energy. Solvents as methanol, ether, or alkaline solutions are toxic and pure water cannot dissolve flavonoid components (Liu *et al.* 2016). Of these solvents, ethanol presents advantages such as being an eco-friendly solvent, a safe solvent, easily available in high purity, completely biodegradable, and mainly safe for human consumption (Shi *et al.* 2005).

The present research examined the impact of the temperature, time, and liquid/solid (L/S) ratio on the yield of extractives from spruce bark using MAE.

EXPERIMENTAL

Materials

Spruce bark characterisation

Spruce bark was kindly supplied by Bioenergo Ltd. (Ruzomberok, Slovakia). The bark was air-dried, ground, and separated into 1.0-mm particle sizes using sieves. The spruce bark was extracted using the accelerated solvent extraction (ASE) method (Sluiter *et al.* 2008), weighed, dried, and analysed to determine the lignin, ash, and holocellulose contents (Table 1). The residual lignin content was determined as Klason lignin (TAPPI T222 om-98 1998), and the extractive content was determined according to Sluiter *et al.* (2008). The ash content was determined using TAPPI T211 om-93 (1993), and the holocellulose was quantified with the sodium chlorite treatment following the procedure by Wise *et al.* (1946).

Table 1. Composition of the Spruce Bark

Component	Content (%)
Holocellulose	52.0 ± 0.2
Lignin	26.4 ± 1.3
Ash	3.6 ± 0.4
Extractives	18.0 ± 1.6

Values represent the average of six replicates ± standard deviation

Methods

Closed-system microwave-assisted extraction

The MAE was performed using a MicroSYNTH Labstation (maximum output = 1.5 kW, frequency = 2.45 GHz, maximum temperature = 250 °C, maximum pressure = 100 bar) (Milestone Inc., Shelton, USA) with a high-pressure 100-mL reactor and 96.6% ethanol as the extracting agent. Three minutes of heat-up time was applied to reach the desired temperature. After MAE, the extracted liquors were cooled to room temperature (maximum cooling time < 15 min) and immediately filtered through Whatman filter paper No. 1 to separate the extract and residue. Extractions were performed with various temperatures (60 °C to 100 °C), irradiation times (1 min to 20 min), and L/S ratios (8 mg/g dry bark to 12 mg/g dry bark).

Yield of extractives

The yield of extractives Y (%) was determined after each experiment by drying the samples at 105 °C to a constant weight. The results were expressed on the basis of the dry matter before and after extraction (Eq. 1),

$$Y\ (\%) = 100 \times m_{\text{extr}} / m_{\text{i}} \qquad (1)$$

where m_{i} and m_{extr} are the mass (g) of the samples before extraction and after extraction and drying, respectively.

Experimental design

The response surface method (RSM) is used to design and develop new processes and to improve existing processes. The RSM represents a set of statistical and mathematical techniques and can create a set of planned experiments that cover all combinations of the design variables.

Table 2. Box–Behnken Design for the Optimization of the MAE

Run	Temperature (°C)	Time (min)	L/S Ratio (mL/g dry bark)	Yield of Extractives (%)
1	60	10.5	12	5.76
2	60	1	10	5.39
3	80	10.5	10	5.82
4	80	10.5	10	6.27
5	100	1	10	6.01
6	100	10.5	8	6.49
7	80	1	12	5.12
8	60	20	10	5.06
9	80	20	12	5.78
10	100	10.5	12	7.66
11	60	10.5	8	6.06
12	80	20	8	5.51
13	100	20	10	6.49
14	80	1	8	5.12
15	80	10.5	10	5.86

Designing the experiment using the Box-Behnken design saved numerous experiments. The experiments were designed for three changing parameters: temperature, time, and liquid to solid ratio (L/S). This method is suitable for the development of a quadratic model. Fifteen experiments were generated by the Box-Behnken design. Two boundary points and one midpoint were selected from the intervals of the changing parameters, and they were coded with the numbers -1, 0, and 1. Table 2 summarizes the experiments. The experiments were run randomly to minimise the effects of uncontrolled variables. An analysis of variance was performed, and a Pareto chart was created to verify the importance of the measured effects.

As shown in Table 2, a 15-run Box-Behnken design was employed with three variables, and the mean amounts of the corresponding compounds extracted from the spruce bark were taken as the responses.

RESULTS AND DISCUSSION

In the present investigation, three main parameters were selected for the optimization of spruce bark extraction. The temperature (60 °C, 80 °C, and 100 °C), extraction time (1 min, 10.5 min, and 20 min), and *L*/*S* ratio (8 mL/g dry bark, 10 mL/g dry bark, and 12 mL/g dry bark) ranges were fixed.

Figure 1 depicts the standardized Pareto chart for the Box-Behnken model. The chart shows the positive and negative effects of the factors on the yield of extractives, which are shown as horizontal bars. The chart indicated that the extraction temperature (A) was the most significant term and had a positive influence on the yield. The other terms with a significant positive influence were the interactions between the time (B) and time (BB), A and A (AA), and A and the L/S ratio (C) (AC).

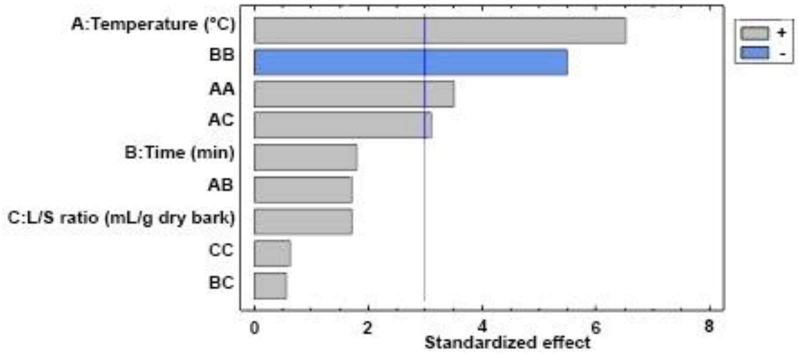

Fig. 1. Standardized Pareto chart for the Box-Behnken Design

The F-test and p-value were used to check the significance of each coefficient (Table 3).

Table 3. Analysis of Variance for the Yield of Extractives

Source	Sum of Squares	Df	Mean Square	F-ratio	P-value
A: Temperature (°C)	2.40	1	2.40	42.80	0.0012
B: Time (min)	0.18	1	0.18	3.21	0.1330
C: L/S ratio (mL/g dry bark)	0.16	1	0.16	2.90	0.1493
AA	0.69	1	0.69	12.30	0.0171
AB	0.16	1	0.16	2.93	0.1478
AC	0.54	1	0.54	9.64	0.0267
BB	1.67	1	1.70	30.29	0.0027
BC	0.02	1	0.02	0.33	0.5931
CC	0.02	1	0.02	0.39	0.5589
Total Error	0.28	5	0.06	-	-

Total (Corr.)	6.34	14	-	-	-

When the p-value is no more than 0.05, the model term is considered significant. The coefficient of determination (R^2) was 0.9558, which demonstrated that the experimental data was in high agreement with the predicted extraction values.

The experimental data were analysed, and an equation to describe the relationship between the yield of extractives and extraction variables was derived (Eq. 2).

$$\mathit{Yield\ of\ Extractives}\ (\%) = 19.546 - 0.2498524\,\mathit{Temperature}\ (^\circ\mathrm{C}) + 0.05327424\,\mathit{Time}\ (\mathrm{min}) - 1.086\,\mathit{L/S\ Ratio}\ (\mathrm{mL/g\ dry\ bark}) + 0.001\,\mathit{Temperature}^2 + 0.001(\mathit{Temperature} \times \mathit{Time}) + 0.009(\mathit{Temperature} \times \mathit{L/S\ Ratio}) - 0.008\,\mathit{Time}^2 + 0.004(\mathit{Time} \times \mathit{L/S\ Ratio}) + 0.019\,\mathit{L/S\ Ratio}^2 \qquad (2)$$

Figure 2 shows that the yield of extractives was affected by the extraction temperature. Boonkird *et al.* (2008) showed that increasing the temperature will change the solution properties, such as the viscosity, surface tension, vapor pressure, and density. These effects are important for changing the extraction efficiency. Figure 1 shows the positive effects of the temperature on the yield of extractives. These results were similar to those of previous reports (Sládková *et al.* 2015; Lazar *et al.* 2016). According to Goula (2013), it should be taken into consideration that a higher temperature (above 60 °C) can lead to thermal degradation of polyphenolic substances. As a result, a faster evaporation of the solvent can lead to a reduction in the extraction efficiency. This temperature effect was not confirmed in this work. The yield of extractives was positively influenced by an increase in both the temperature and L/S ratio.

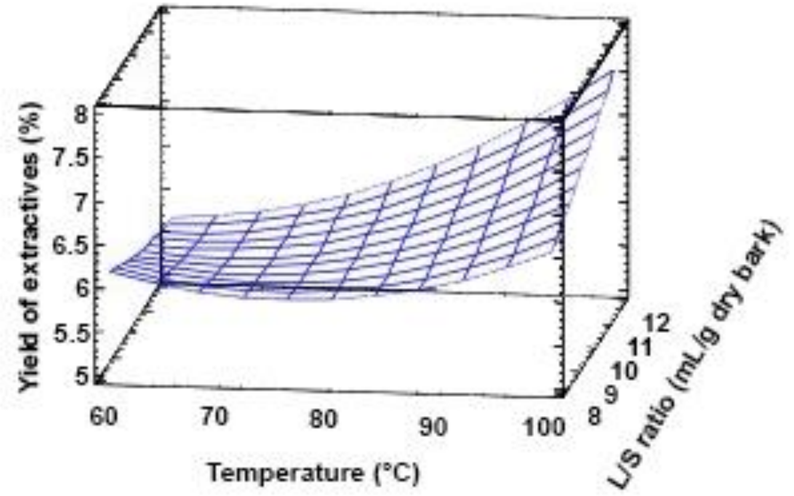

Fig. 2. Predicted contour levels for the yield of extractives as a function of the temperature and L/S ratio at a constant extraction time

The reports in the literature state that the *L*/*S* ratio plays an important role in the extraction process for obtaining phytonutrients. Figure 3 shows that increasing the L/S ratio increased the yield of extractives. At a constant temperature of 80 °C, there were limits with the extraction time. The time had positive effects on the yield from 0 min to 12 min.

The measured yield of extractives obtained under the different extraction conditions is represented as the contour surface in Fig. 4. This function has a typical saddle shape. It was observed from the contour surface that at a constant *L*/*S* ratio (10 mL/g dry bark) the yield of extractives depended on the extraction temperature and time.

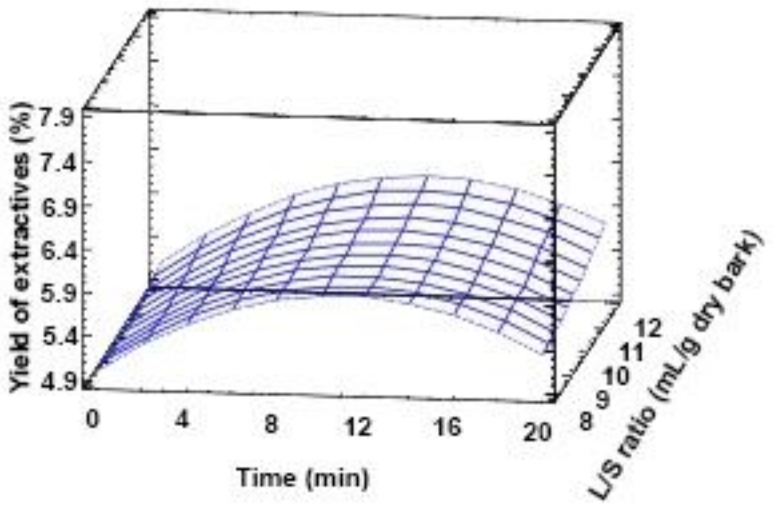

Fig. 3. Predicted contour levels for the yield of extractives as a function of the extraction time and L/S ratio at a constant temperature

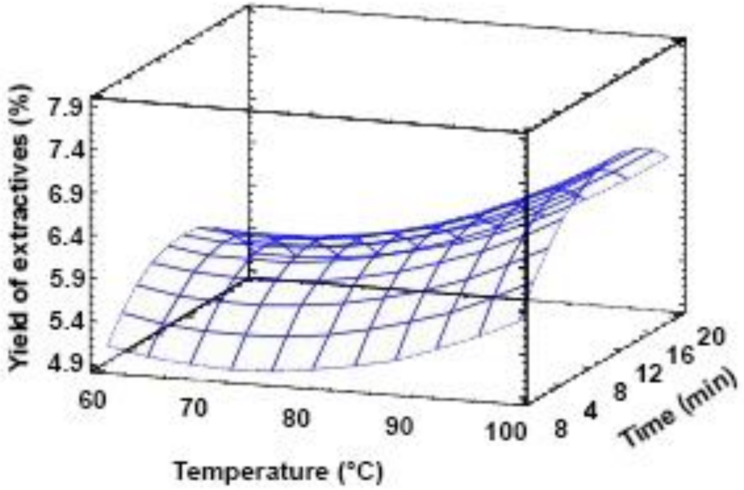

Fig. 4. Predicted contour levels for the yield of extractives as a function of the temperature and extraction time at a constant *L*/*S* ratio

The temperature had positive effects on the yield from 80 °C to 100 °C, at an extraction time of 1 min to 14 min and a constant *L*/*S* ratio of 10.0 mL/g dry bark. A further increase in the time caused a significant decrease in the yield of extractives.

The regression analysis indicated that a maximum extraction yield of 7.61% of the spruce bark was reached with the following optimum conditions: *L*/*S* ratio of 12.0 mL/g dry bark, temperature of 100 °C, and time of 13.4 min. The model was validated 3 times with yield of extractives of 7.28%, 7.42%, and 7.51%. The average yield of extractives (7.40%) is consistent with the predicted values. This further confirms that the model is well fitted with the experimental data.

Comparison of different extraction procedures

To evaluate the efficiency of the MAE method, different extraction procedures were compared (Ház *et al.* 2016). Four extraction techniques were selected: MAE (96.6% ethanol, 10.5 min to 20 min, 60 °C to 80 °C, and 2.45 GHz), ASE (96.6% ethanol, 35 min to 38 min, and 80 °C to 160 °C; and 99.7% ethanol, 15 min, and 80 °C to 180 °C), Soxhlet extraction (96.6% ethanol and 540 min), and supercritical solvent extraction (SFE; supercritical CO_2 and ethanol, 60 min, 60 °C to 140 °C, and 7000 psi; and supercritical CO_2 and ethanol, 30 min, 70 °C, and 3770 psi). The extraction yields were determined and are listed in Table 4.

Garcia-Ayuso *et al.* (1998) found that by applying microwave irradiation to Soxhlet extraction, an 8-h extraction could be reduced to between 50 min and 60 min. Therefore, the expectation was that MAE should be able to extract extractives as effectively as the Soxhlet extraction in a shorter period of time. The composition and extractives content from wood materials (*i.e.*, bark) depend primarily on the solvents used and especially on the extraction conditions.

Table 4. Yield of Extractives from Spruce Bark using Various Procedures

Extraction Method	Conditions and Solvents	Yield of Extractives (%)	Reference
Soxhlet	540 min, 96.6% ethanol	10.2 ± 0.2	Jablonský *et al.* (2015b)
MAE	100 °C, 20 min, 20 mL/g L/S ratio, 96.6% ethanol, 2.45 GHz	6.49 ± 0.01	Sladkova *et al.* (2016)
	60 °C, 20 min, 20 mL/g L/S ratio, 96.6% ethanol, 2.45 GHz	5.06 ± 0.01	Sládková *et al.* (2016)
	80 °C, 20 min, 16 mL/g L/S ratio, 96.6% ethanol, 2.45 GHz	5.51 ± 0.01	Sládková *et al.* (2016)
	60 °C, 10.5 min, 16 mL/g L/S ratio, 96.6% ethanol, 2.45 GHz	6.06± 0.01	Sládková *et al.* (2016)
	60 °C, 10.5 min, 24 mL/g L/S ratio, 96.6% ethanol, 2.45 GHz	7.66 ± 0.01	Sládková *et al.* (2016)
ASE	35 min, 80 °C, 1500 psi, 96.6% ethanol	7.5 ± 0.2	Jablonský *et al.* (2015b)
	36 min, 120 °C, 1500 psi, 96.6% ethanol	10.5 ± 0.3	Jablonský *et al.* (2015b)
	38 min, 160 °C, 1500 psi, 96.6% ethanol	13.8 ± 0.2	Jablonský *et al.* (2015b)
	15 min, 99.7% ethanol, 80 °C	22.4	Co *et al*. (2012)
	15 min, 99.7% ethanol, 130 °C	25.8	Co *et al.* (2012)
	15 min, 99.7% ethanol, 180 °C	34.8	Co *et al.* (2012)
SFE	60 min, 60 °C, 7000 psi, $scCO_2$ + 96.6% ethanol	6.39 ± 0.01	Ház *et al.* (2016)
	100 min, 100 °C, 7000 psi, $scCO_2$ + 96.6% ethanol	6.68 ± 0.01	Ház *et al.* (2016)
	140 min, 140 °C, 7000 psi, $scCO_2$ + 96.6% ethanol	8.46 ± 0.01	Ház *et al.* (2016)
	30 min, 70 °C, 3770 psi, $scCO_2$ + 99.7% ethanol (80:20)	3.3	Co *et al.* (2012)

$scCO_2$ – supercritical CO_2

The results indicated that the highest yield was achieved by ASE (Co *et al*. 2012).

This method uses high pressure, which keeps the solvent in the liquid phase even at higher temperatures and facilitates the penetration of the solvent into the sample. The extraction time is relatively short, but the preparation of the sample in the extraction cells takes some time. One of the disadvantages of ASE is that the amount of solvent used is irreproducible (Giergielewicz-Możajska *et. al* 2001). The MAE and SFE processes appeared to be promising alternatives to classic methods because they are faster and use less solvent compared with conventional methods. Supercritical solvent extraction has the advantages of a low temperature, reduced energy consumption, and higher product quality because of the absence of solvent in the final phase of the solute. Supercritical carbon dioxide is recognized as the ideal solvent for the extraction of bioactive compounds that are non-toxic, non-explosive, and easy to remove from the final extract (Espinosa-Pardo *et al*. 2014). Based on these findings (Table 4), it was concluded that MAE is more efficient and faster than both the Soxhlet extraction and SFE methods because of the elevated temperature and pressure.

During MAE, heat is dissipated volumetrically inside the irradiated medium. The direction of heat and mass transfer are identical in MAE, which further enhances the extraction yield (Mishra and Aeri 2016). During conventional extraction, heat is transferred from the heating medium to the inside of the sample.

CONCLUSIONS

1. In this study, extraction from spruce bark with the MAE method was conducted.
2. The effect of the extraction parameters, including the microwave power, *L/S* ratio, and extraction time, were evaluated by the Box-Behnken method.
3. The maximum yield obtained with the Box-Behnken design was 7.61% for the optimum extraction parameters (temperature = 100 °C, time = 13.4 min, and *L/S* ratio = 12.0 mL/g).

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