SUPPORT VECTOR MACHINE COMBINED WITH GENETIC ALGORITHM FOR OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF POLYPHENOLS FROM SPRUCE WOOD BARK

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We investigated a simple and rapid microwave-assisted extraction (MAE) procedure that was optimized for extraction of polyphenols from spruce wood bark. Important variables that can potentially affect the extraction efficiency, namely temperature, ethanol concentration and extraction time, were optimized using support vector machines and an evolutionary algorithm. Experiments were conducted in this study towards the construction of a modeling technique. The optimum conditions obtained include: ethanol concentration of 50%, extraction time of 3 minutes and temperature of 60 °C, which led to the total polyphenols content (TPC) of 58.25 mg gallic acid equivalents (GAE g^{-1} of spruce bark tested).

Keywords: phenolic compounds, waste, temperature extraction, antioxidant, ethanol concentration

INTRODUCTION

Over the past 10 years, researchers have become increasingly interested in various physiological activities of polyphenols due to their relationship with human health, because they can protect the human body from free radicals and inhibit oxidation processes.[1-4] From the point of view of biological activity, polyphenols are considered as compounds with a perspective of being isolated in their pure form from natural sources, and as having higher antioxidant activity than that of conventional antioxidants, such as vitamins C, E or β carotene. Phenolic compounds are produced as secondary metabolites, which are widely encountered in plant tissues, and are recognized as bioactive ingredients of foods, promoting human health.[5,6] The main reason for the interest polyphenols have raised is the recognition of their antioxidant properties, their great abundance in human diet, and many biologically significant functions, such as the prevention of various diseases associated with oxidative stress, neurodegenerative and cardiac diseases.[7]

Tree barks together with other wood wastes are low-value by-products[8] in the forest industry and in pulp production,[9,10] and are usually used as an energy source through incineration. Tree bark was found to be rich in health-promoting compounds. Prior to incinerating the bark, it would be worthwhile first extracting valuable antioxidants. Tree bark is a rich source of secondary metabolites and contains several compounds with biological activity, which present commercial interest and can be used in different fields.[11,12]

Conventional methods for the extraction of phenolic compounds are usually based on solvent extraction, using ethanol or methanol solvents for more polar antioxidants, and other solvents, such as chloroform or dichloromethane, for less polar antioxidants. These extraction methods are carried out at ambient temperature or at the boiling point of the solvent used and are laborious and time-consuming.[13]

The extraction of polyphenols is one of the critical steps in achieving complete recovery of valuable compounds.[14] Many factors contribute to the efficiency of solvent extraction, such as the type of the solvent, its concentration, the pH, extraction temperature/time, pressure and particle size of the raw material. Conventional techniques, such as Soxhlet extraction, have been used to isolate phenolic compounds.[15] The main disadvantages are the small amount of phenolic compounds caused by oxidation, ionization and hydrolysis during extraction, as well as the long extraction time and high quantity of solvent used.[16] Other techniques, which include microwave-assisted extraction and ultrasonic-assisted extraction, are two promising methods, recognized as economical (less solvent used, shorter time extraction), simple, with a high rate and efficiency, as well as offering increased quality of the extract, without altering the antioxidant properties.[17-20]

In the extraction of bioactive compounds, the microwave-assisted method (MAE) represents a remarkable technique because the process uses microwave energy to heat the solvents in contact with the sample rapidly and efficiently, and the direct interaction of microwaves with the free water molecules present in the vascular system gives better extraction yields.[21,22] MAE has attracted special interest and has been widely used in different fields for separating interesting components from a wide variety of sample matrices, such as natural product, food and agricultural wastes.[23,24] The principle of homogeneous heating using microwaves is based on the direct action of the electromagnetic radiation on the molecules through ionic conduction and dipole rotation, resulting in heating.[25] The major advantages of this method are the reproducibility and applicability of the method to various sample sizes, the dramatic reduction in time needed to perform highly efficient extractions, and the efficient extraction of polar organic compounds.[26]

Typical parameters that affect microwave assisted-extraction include the solvents, the matrix and the extraction time, and are described below.

It has been demonstrated that the amount of water present in the solvent (*i.e.* the concentration of the aqueous solution) significantly influences the extraction yield. An aqueous solution of a certain organic solvent is desired for certain extractions, as the presence of water would improve the penetration of the solvent into the sample matrix and thus will enhance the heating efficiency.[25] Ethanol is most frequently used, being an excellent microwave-absorbing solvent, which is suitable for extracting many active compounds from many plants. In order to get optimum extraction yields, researchers even use mixtures of high and low microwave-absorbing solvents.

As regards the matrix, the solvent ratio plays an important role in microwave-assisted extraction.[27] The solvent volume must be sufficient for the entire sample to be immersed completely in the solvent, so that the material can swell during the irradiation process. In conventional extraction methods, a higher ratio of solvent volume to solid matrix gives better extraction yields, whereas in the case of MAE, a higher solvent:matrix ratio may not give a better yield because of non-uniform distribution and exposure to microwaves.[28]

The extraction time is another important factor that influences the extraction process of MAE. The quantity of polyphenolic compounds extracted can be increased with an increase in the extraction time, but there is an associated risk for thermo-labile compounds.[29] Varying the time periods is necessary for the extraction of different matrices, but exposure of even a few seconds has been demonstrated to give excellent yields. However, extraction time optimization is influenced by the dielectric properties of the solvent.

Many reports have been published on the application of MAE of secondary metabolites from plants or waste products as a promising alternative sample preparation technique.[30] Some examples are extractions of total phenolic acids from mandarin peels,[31] antioxidants from *Citrus limon* residues[32] and polyphenols from waste peanut shells.[4] Total phenolics were extracted from aromatic plants, such as *Rosmarinus officinalis*, using microwave-assisted extraction. Raman and Gaikar[33] investigated the extraction of piperine from powdered black pepper by a conventional method and by microwave irradiation.

Conventional extraction for one hour led to 20% recovery of target compounds, compared to microwave irradiation, which yielded 80% in 2 minutes. Compared to traditional reflux extraction, microwave-assisted extraction is a technique that promotes cutting down the extraction time, decreasing solvent consumption and increasing extraction yields. It has also been applied for the extraction of natural compounds from food stuffs, *e.g.* polyphenol compounds from tea,[34] grape seeds[35] and caffeine. Nayac and coworkers extracted polyphenols from peels of *Citrus sinensis*, using MAE and the results were compared with those of conventional, ultrasound-assisted and accelerated solvent extraction. Thus, total phenolic contents of 12.09 mg GAE g^{-1}, 10.35 mg GAE g^{-1}, 6.26 mg GAE g^{-1} dry weight were recovered by MAE, UAE and ASE.[36] For industrial production of antioxidants, it could be opportune environmentally and economically sustainable to use biomass waste from forestry/agricultural industry as feedstock.

However, the feasibility of using microwave for the extraction of phenolic compounds from spruce bark has not been explored yet. The challenge is the high-yield and energy-efficient extraction of these compounds. The main aim of this study is to optimize the microwave-assisted extraction of phenolic compounds from spruce bark using the support vector machine method (SVM) and evolutionary algorithms (EA). The influence of three factors, including extraction temperature, time and ethanol concentration on the extraction yields of total phenolic compounds was investigated.

We have to take into account the interdependency between the considered parameters. It is well known that the interaction between experimental parameters can lead, in the optimization procedure, to values different from those resulted when considering each individual parameter. In addition, in the article, four optimization cases are solved, with the imposed (limited) domains of experimental values with the goal to save energy and materials, as well as to avoid undesired phenomena (degradation, destruction). Under these conditions, the optimization results could be different from those obtained from the analysis of individual parameters. The optimization also has the goal to find optimal conditions for practical applications.

Support vector machines (SVMs)[37] represent a method of classification (binary classification in the standard approach) and regression. A SVM model considers the training instances as points in a multi-dimensional space, which can be transformed in order for the classes to be separated with a large margin. The idea of splitting the hyperspace in two parts can be also found in the training principle of the single-layer perceptron, for example, but, in this case, it works only if the problem is linearly separable. For the non-linear cases, SVM uses kernels for mapping the data into a different space with more dimensions compared to the original space, where a problem can become linearly separable even if it was not originally so. In addition, some errors in the classification of the training data can be allowed using soft margins with the goal of increasing the generalization capability.

SVM benefits from solid mathematical foundations, which offer very good accuracy, compared to other learning methods. Another advantage is the small number of parameters that the user has to choose from (the type of kernel with its parameters and a cost parameter, which defines the balance between tolerance for training errors and generalization capability). A small disadvantage is the fact that the standard model is binary and, in order to apply it to problems with multiple classes, it is necessary to obtain several partial models, subsequently aggregated based on various strategies, such as "one-versus-all" or "one-versus-one". Nevertheless, support vector machines represent a state-of-the-art classification technique that has been intensively studied and benchmarked against a variety of classification methods, proving both theoretical and computational advantages.[38,39]

The support vector machine method has several advantages over other learning techniques. SVM is based on the structural risk minimization principle from computational learning theory, which always converges to a global optimum, in contrast with the empirical risk minimization of the classical neural networks. Additionally, SVM has strong generalization capabilities. As a disadvantage, SVM models are computationally expensive; they need time and memory as the complexity of the model increases (depending on the dimension of the training data).

In this work, MAE parameters, such as ethanol concentration, extraction temperature and extraction time, were optimized by SVM and GA methodology in order to obtain the optimal extraction yield of polyphenols from spruce wood bark. Since there are no reports on microwave-assisted extraction of polyphenols from spruce wood bark, this study was designed to demonstrate the utility of microwave-assisted extraction in the determination of the total phenolic contents of spruce wood bark.

EXPERIMENTAL

Materials

Spruce wood bark of industrial origin was purchased from the timber company "Alpine" LTD, Vatra Dornei, Romania. After drying at room temperature and under normal aeration, the spruce bark was milled (0.5-1 mm). Ethanol, Folin Ciocalteu's phenol reagent, gallic acid standard and sodium carbonate (Na_2CO_3) were provided by Sigma-Aldrich and Fluka. All solvents used were of analytical grade. Distilled water was used for all experiments.

Microwave extraction method

Microwave-assisted extraction experiments were performed using a Milestone Microwave Lab station START S, with an infrared automatic temperature control IRTC-500. The working microwave power was set to 300 W to investigate the influence of aqueous ethanol concentration, extraction time and temperature. The spruce bark (1 g) was placed into a 30 mL volumetric flask and made up to volume with aqueous ethanol solvent. Experiments were carried out to determine the effect of extraction time (1-55 min), ethanol composition of the solvent (ethanol:water 30-80%) and temperature (30-60 °C) on MAE efficiency. The ground bark sample was extracted and the slurry obtained was filtered through Whatmann No. 1 filter paper. The filtrate was collected and allowed to cool at room temperature, then immediately used for determining the total phenolic content.

Determination of total polyphenol content

The total polyphenolic content (TPC) in the extracts was determined spectrophotometrically using Folin-Ciocalteu's reagent, by a previously developed protocol.[40] The calibration curve was made with standard solutions of gallic acid and measurements were carried out at 765 nm. The total polyphenolic concentration was expressed in mg gallic acid equivalents g^{-1}. Sample (1 mL), FC reagent (0.5 mL), 10% saturated sodium carbonate solution (2 mL) and 5 mL distilled water were added. The absorbance was measured at 765 nm using a UV-visible spectrophotometer (CINTRA UV-260) after 90 minutes of incubation in the dark at room temperature. The results are expressed as g gallic acid equivalents L^{-1} (g GAE L^{-1}). The calibration curve, having the equation y = 0.006x + 0.0377, where y is the absorbance of sample and x is the sample concentration, with the determination coefficient, R^2 = 0.9989, was used. All the measurements were taken in triplicate.

Support vector machines

SVMs are presently among the best available methods for classification and regression. In their standard formulation for classification, they build a model on the training set comprised of *N*-dimensional vectors or points $x_i \in \Re^N$. The desired output results, *i.e.* the class of an instance x_i, are the corresponding $y_i \in \{-1, 1\}$. The fundamental idea of SVM is to find a separating hyperplane between the two classes, such as the distance (or the margin) between the classes should be maximized. The separating hyperplane has the equation w · x + *b* = 0, and thus the decision function is:[31]

$$f(\mathbf{x}) = sign\,(\mathrm{w} \cdot \mathrm{x} + b) \quad (1)$$

The idea of maximizing the separation margin is rooted in the mathematical theory of statistical learning, which proves that the best generalization performance is ensured in this way. The closest points on both sides of the separating hyperplanes are called "support vectors".

More specifically, finding the optimal hyperplane involves solving the following quadratic optimization problem:

$$\text{Minimize } f(x) = \frac{\|\mathbf{w}\|^2}{2} \quad (2)$$

with the constraints:

$$g_i(x) = y_i(\mathbf{w} \cdot x_i) - 1 \geq 0, i = 1...N \quad (3)$$

Since SVM can handle problems with high dimensionality *N* (possibly infinite), a better way to solve equation (2) is to consider the dual problem, using the corresponding Lagrangian function. The dual problem is often easier because the Lagrange multipliers are all 0, except for the ones associated with the support vectors.[41]

If the initial data are not linearly separable, they can be transformed into a higher-dimensional space using feature mapping:

$$\Phi : \Re^N \to \Re^F \quad (4)$$

and in that space the data can become linearly separable. Since all the computations involve dot products of vector pairs, a kernel function is usually employed:

$$K(\mathbf{x}, \mathbf{y}) = \Phi(\mathbf{x}) \cdot \Phi(\mathbf{y}) \quad (5)$$

Commonly used kernel functions are polynomial kernels and radial basis function (RBF) kernels.

In the case of non-separable classes, one can control the trade-off between allowing errors in the classification or striving for better accuracy at the expense of generalization capacity, using the cost parameter *C*.

The most commonly used algorithm for solving the optimization problem is the sequential minimal optimization (SMO) proposed by X. Zhou *et al.*[42] It decomposes the overall quadratic programming problem into sub-problems involving only 2 multipliers. This approach greatly reduces memory and

CPU time and thus can be applied to solve real-world problems with a large number of training data.

In our experiments, the SVM-light software was used.[43]

Evolutionary algorithms

There are many optimization situations where multiple local optima exist and the task is to find the global optimum. An evolutionary algorithm (EA) is an optimization technique inspired from the biological natural selection. It is based on a population of individuals (chromosomes), *i.e.* potential solutions, whose degree of adaptation, or quality, is given by a so-called "fitness function", which defines the objective of the optimization problem. Individuals with better fitness values have more chances to reproduce. Depending on the nature of the optimization problem, many encoding options are available, *e.g.* binary, real-valued, permutation-based, random key encoding *etc*.[44]

The main operators of an evolutionary algorithm are: selection, crossover and mutation. Selection is the process of selecting two parents for reproduction, taking into account that individuals with a higher quality should have more chances of reproduction. Again, there are several selections methods commonly used, *e.g.* roulette-wheel, rank-based or tournament selection. After two parents have been selected, their genes are combined through crossover, which gives a child a part of the genome of one parent, and the rest from the other. Finally, before being inserted into the population of the next generation, mutation can occur, which changes a small number of genes in the child. Several variations exist for each of these operators.[45]

The main steps of an EA are presented below:

- Initialization: the genes of the individuals are randomly initialized with values in their allowed domains;
- Until a stopping criterion is met (*e.g.* a maximum number of generations or a convergence condition):
    - Select parents for reproduction;
    - Create a child (or two) by crossover;
    - Apply mutation to the child (children);
    - Introduce the child (children) into the new population.

The main advantages of EAs are: the ability to handle problems where differential-based techniques are difficult or even impossible to use (*e.g.* discontinuous problems), the use of parallelism to increase the chances of finding the global optimum and overall simplicity. Their main disadvantage lies in their rather high computational effort and sometimes their inability to provide good solutions is a short time. Another difficulty is that the user has a wide range of parameters that should be tuned in order to have better performance for the problem at hand.

RESULTS AND DISCUSSION

Effect of extraction temperature on polyphenol extraction

Microwave-assisted extraction (MAE) combines fast heating in the microwave field with traditional solvent extraction.

Different extraction temperatures were set to 30, 35, 40, 45, 50 and 60 ºC, while the other reaction conditions were set as follows: extraction time of 3 minutes, aqueous ethanol to 50% (v/v).

The effect of extraction temperature on the yield of total polyphenols extracted from spruce bark is shown in Figure 1. It demonstrates that the extraction yields of spruce bark significantly increased when the temperature was raised from 30 to 60 ºC.

The present results reveal that the highest yield of phenolic compounds was obtained with the value of 51.33 mg GAE g^{-1} when the sample was extracted at 60 °C. The polyphenols content crossed from 45.02 mg GAE g^{-1} spruce bark at 40 °C to 51.33 mg GAE g^{-1} spruce bark at 60 °C. Higher values obtained for total phenolic content at higher temperatures were expected, as a higher temperature permits better penetration of the solvent into the spruce bark matrix and higher solubility of polyphenols in the solvent. The increasing temperature to 60 °C may improve the release of compounds from the matrix, and thereby, the availability of total phenolic compounds increased. Increasing temperature improved extraction efficiency due to the increased diffusivity of the solvent. Moreover, in the open-end microwave vessel used in this study, the temperature of the solvent could quickly reach the point of the set temperature. S. Bianchi *et al.*[46] successively extracted phenolic compounds from Norway spruce bark increasing the extraction temperature from 30 to 150 °C in steps of 15 °C. They observed that the total yield in the first extraction step at 30 °C was substantially higher than the following steps up to 105 °C. Over 105 °C, the total yield again remarkably increased. The amount of total phenolics, as detected by the Folin-Ciocalteu assay, remained almost constant along the extraction steps. In the range of temperature between 30 and 105 °C, the phenolics in the extracts were more preponderant, while beyond these boundaries, a more relevant extraction of carbohydrates occurred. They concluded that extraction temperatures over 100 °C are not recommended, because of the high amount of polysaccharides (from the degradation of hemicelluloses) and highly condensed phenolic oligomers in the product.

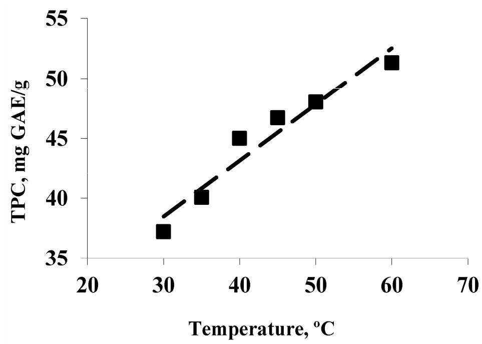

Figure 1: Effect of different temperatures on total polyphenol content (other conditions: 50% aqueous ethanol solution and 3 min extraction time)

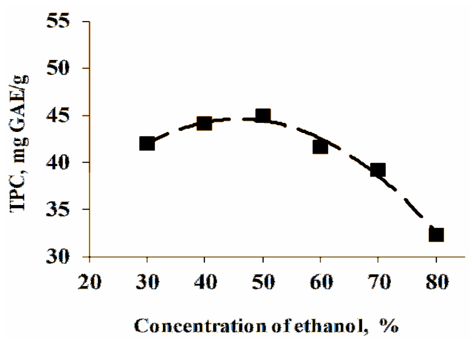

Figure 2: Effect of different ethanol:water ratios on total polyphenol content (other conditions: 40 °C and 3 min)

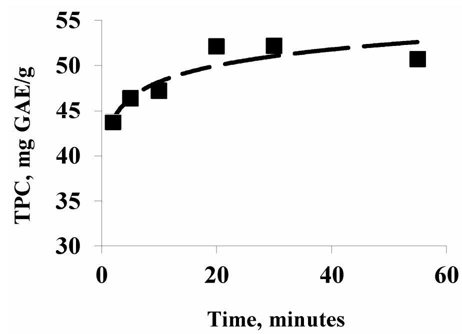

Figure 3: Effect of extraction time on total polyphenol content (other conditions: 50% EtOH and 40 °C)

Effect of ethanol concentration on the extraction of polyphenols

Ethanol is usually preferred in practice due to its several advantages: it is a non-toxic and inexpensive solvent. For these reasons, ethanol was chosen for all experiments to determine the effect of its different concentrations in water on the efficiency of microwave extraction. Different ethanol concentrations were prepared, such as 30, 40, 50, 60, 70 and 80%, v/v in order to investigate the influence of ethanol concentration on the recovery of total phenolic compounds from spruce bark, when the other reaction conditions were set as follows: microwave power of 300 W, extraction time of 3 min, 40 °C temperature and ratio of liquid to solid of 30 mL g^{-1}. Figure 2 shows that the extraction of TPC was greatly influenced by the ethanol concentration in water. The most suitable concentration to extract the highest content of polyphenols, of 45.02 mg GAE g^{-1} spruce bark, was that of 50% aqueous ethanol.

As can be seen, the best yield was obtained by 50% aqueous ethanol solution (45.02 mg GAE g^{-1} spruce bark), followed by 40% aqueous ethanol solution. When ethanol concentration increased from 30% to 50%, v/v, the total phenolic content of the extracts crossed from 42.03 to 45.02 mg GAE g^{-1} of spruce bark. The higher dielectric constant of the 50% aqueous ethanol mixture helps in absorbing the microwave energy, thus increasing the extraction efficiency and the release of total polyphenols into the extract. When the ethanol concentration was higher than 60%, v/v, the extraction slowly decreased, as getting close to pure ethanol. So, the application of water combined with other organic solvents induces the creation of a moderately polar medium, ensuring the optimal conditions for the extraction of polyphenols. Using water in combination with ethanol leads to an increase in swelling of plant materials and the contact surface area between the plant matrix and the solvent, improving the extraction yield.[17] Amirah *et al.*[47] reported the optimal conditions for MAE of gallic acid from stem bark of *Jatropha curcas* as 50% ethanol concentration, extraction time of 2 min, temperature of 40 °C. The solvent molecules may absorb the microwave energy and become polarized.

Effect of extraction time on total polyphenol content

The recovery of TPC affected by different extraction time is shown in Figure 3. The extraction procedures were repeated by varying the extraction time from 3 to 55 minutes, while the other four factors – microwave power, ethanol proportion, ratio of liquid to solid and temperature – were fixed at 300 W, 50%, v/v, 30 mL g^{-1} and 40 °C, respectively. There was a positive linear correlation between total phenolic content and extraction time. The results indicate that the recovery of TPC increased with the increase of MAE time of extraction. The recovery could reach its maximum of 52.16 mg GAE g^{-1} spruce bark in 20 minutes during the MAE process.

Prolonged exposure involves the risk of degradation by heating. Similarly, in the current study, a consistent fall in the extraction yield after 30 min of exposure to microwaves is observed. Also, similar results to those of this study were obtained in extracting polyphenols from flaxseed[48] and green tea leaves.[49] In addition, C. Y. Guo *et al.*[50] investigated the effects of ethanol concentration, extraction temperature and duration of microwave extraction on the flavonoids from *Inula helenium* and the optimal conditions were found to be as follows: ethanol concentration of 50%, v/v, extraction time of 240 s and extraction temperature of 60 °C. M. Co *et al.*[13] studied conventional extraction of antioxidants from spruce (*Picea abies*) bark with ethanol under ambient conditions. Compared with other techniques, such as pressured fluid extraction (PFE), they obtained a lower yield and a lower capacity (18.0 wt% in 24 hours, compared to 22.4 wt% in 15 min at 80 °C).

Other experiments were performed to reveal the effect of different ethanol concentration on total polyphenols content in spruce bark as $40 < T < 60$ °C.

Figure 4a and b shows how the extraction rate of total polyphenols decreases from 50% to 70% ethanol concentration. Microwave-assisted extraction with 50% aqueous ethanol was found to give a higher yield of the extract than 70% aqueous ethanol.

Water and low concentration of ethanol can access cells, but a high concentration of ethanol can cause denaturation of polyphenols, affecting the extraction rate. The possible reason for the increased efficiency is the increase in swelling of the plant material caused by water, which enhances the contact surface area between the plant matrix and the solvent.[11] It can be noticed that with increasing extraction time from 30 minutes (52.16 mg GAE g^{-1} spruce bark) to 55 minutes (50.7 mg GAE g^{-1} spruce bark), no significant increase or decrease in extraction efficiency occurs.

Modeling the microwave-assisted extraction process using SVMs combined with GA

A support vector machine model was created to approximate the experimental data. The inputs of the model were temperature, ethanol concentration and time, and the output was considered polyphenol concentration. In this way, the modeling technique has the goal to predict the final concentration of polyphenols as a function of the working conditions.

The SVM model with the best performance used a radial basis function kernel:

$$K(\mathbf{x},\mathbf{y}) = \exp\left(-\|\mathbf{x}-\mathbf{y}\|^2\right) \qquad (6)$$

and the value of the cost parameter *C* was 10000.

Figure 5 shows the comparison between experimental and simulation results; a good agreement is demonstrated by the value of determination coefficient $R^2 = 0.9427$.

The developed SVM model was included in an evolutionary algorithm (genetic algorithm, GA) optimization procedure, which had the goal of obtaining maximum polyphenol concentration. The SVM-EA optimization method works as follows:

- A new chromosome is initialized or evolved, with three real-valued genes, corresponding to temperature, ethanol concentration and extraction time;
- The corresponding fitness function is computed by applying these three values as inputs to the network; the fitness function is the output of the network, polyphenols concentration;
- The chromosome is further processed by the evolutionary algorithm operators.

The performance of the GA algorithm depends on the control parameters: dimension of initial population (pop_dim), number of generations (gen_no), crossover probability (cross_prob) and mutation probability (mut_prob). Different values for the control parameters were tested in the optimization, through the trial and error method.

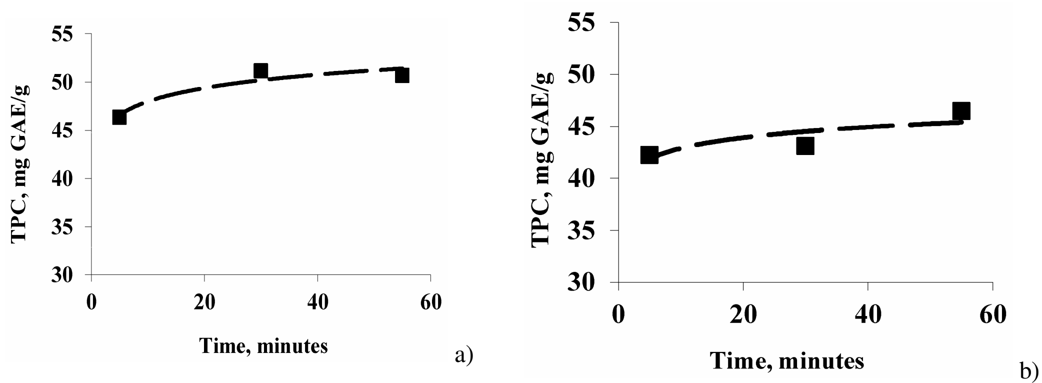

Figure 4: Effect of extraction time at 40 °C and 50% aqueous ethanol solution (a) and at 40 °C and 70% aqueous ethanol solution (b) on total polyphenol content

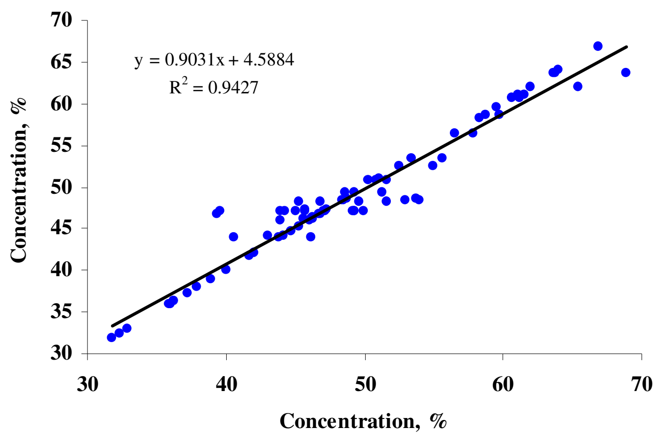

Figure 5: Experimental and SVM results for polyphenol concentration

The maximum intervals considered for the decision variables were: temperature, T = 30-64 °C, aqueous ethanol solution = 30-80%, v/v, and extraction time, t = 1-120 min. This was referred as Case 1. Other three problems were formulated restricting the domains of values for the decision variables with the goal to force the optimization results to be situated into convenient experimental domains. Thus, Case 2 is: T = 30-64 °C, aqueous ethanol solution = 30-80%, and t = 1-15 min; Case 3 is: T = 30-45 °C, aqueous ethanol solution = 30-80%, and t = 1-120 min; Case 4 is: T = 30-45 °C, aqueous ethanol solution = 30-80%, and t = 1-15 min. Polyphenols concentration, TPC, was recorded each time.

Table 1 presents several optimization results for the above formulated cases and different values for the control parameters of EA.

Looking at EA parameters and Case 1, one can see that an increase of pop_dim and gen_no determines obtaining a greater TPC. With pop_dim = 50 and gen_no = 150, TPC = 63.69, maximum values are listed in Table 1. The other two parameters, cross_prob and mut_prob do not have any significant influence on the optimization results.

The restrictions of the cases noted 2, 3, and 4 imposed a shorter time (Case 2 and Case 4) or a lower temperature (Case 3 and Case 4), which led to a maximum polyphenol concentration. In these situations, the maximum TPC was around 58 mg GAE g^{-1}, obtained with T = 60 °C, t = 3 min, and concentration of ethanol = 50%.

Table 1
Optimization results obtained for the four optimization cases

No.	EA parameters	Case 1	Case 2	Case 3	Case 4
1	pop_dim = 20 gen_no = 20 cross_prob = 0.95 mut_prob = 0.95	T = 42.51 °C Aqueous ethanol solution = 42.56% t = 25.48 min TPC = 48.17 mg GAE g^{-1}	T = 48.77 °C Aqueous ethanol solution = 49.61% t = 4.20 min TPC = 48.39 mg GAE g^{-1}	T = 41.98 °C Aqueous ethanol solution = 68.23% t = 16.66 min TPC = 48.17 mg GAE g^{-1}	T = 31.04 °C Aqueous ethanol solution = 65.21% t = 7.72 min TPC = 48.17 mg GAE g^{-1}
2	pop_dim = 50 gen_no = 20 cross_prob = 0.95 mut_prob = 0.05	T = 49.88 °C Aqueous ethanol solution = 70.52% t = 30.12 min TPC = 51.38 mg GAE g^{-1}	T = 60.32 °C Aqueous ethanol solution = 50.11% t = 4.28 min TPC = 50.09 mg GAE g^{-1}	T = 41.46 °C Aqueous ethanol solution = 40.81% t = 85.37 min TPC = 48.17 mg GAE g^{-1}	T = 44.67 °C Aqueous ethanol solution = 43.11% t = 14.78 min TPC = 48.17 mg GAE g^{-1}
3	pop_dim = 100 gen_no = 20 cross_prob = 0.95 mut_prob = 0.05	T = 60.14 °C Aqueous ethanol solution = 70.31% t = 29.55 min TPC = 57.29 mg GAE g^{-1}	T = 59.97 °C Aqueous ethanol solution = 49.84% t = 2.99 min TPC = 58.07 mg GAE g^{-1}	T = 39.88 °C Aqueous ethanol solution = 49.48% t = 54.83 min TPC = 48.39 mg GAE g^{-1}	T = 41.33 °C Aqueous ethanol solution = 70.65 % t = 10.34 min TPC = 48.17 mg GAE g^{-1}
4	pop_dim = 100 gen_no = 100 cross_prob = 0.95 mut_prob = 0.05	T = 60.01 °C Aqueous ethanol solution = 50.01% t = 30.1 min TPC = 63.81 mg GAE g^{-1}	T = 59.95 °C Aqueous ethanol solution=49.86 % t = 3.01 min TPC = 58.12 mg GAE g^{-1}	T = 37.36 °C Aqueous ethanol solution = 63.64 % t = 44.63 min TPC = 48.17 mg GAE g^{-1}	T = 33.40 °C Aqueous ethanol solution = 36.98 % t = 11.20 min TPC = 48.17 mg GAE g^{-1}
5	pop_dim = 50 gen_no = 150 cross_prob = 0.95 mut_prob = 0.05	T = 59.98 °C Aqueous ethanol solution = 49.99% t = 35.01 min TPC = 63.69 mg GAE g^{-1}	T = 60.01 °C Aqueous ethanol solution = 49.91% t = 2.99 min TPC = 58.25 mg GAE g^{-1}	T = 39.93 °C Aqueous ethanol solution = 49.96% t = 55.16 min TPC = 48.47 mg GAE g^{-1}	T = 43.04 °C Aqueous ethanol solution = 36.23% t = 3.04 min TPC = 48.17 mg GAE g^{-1}

CONCLUSION

The microwave-assisted extraction technique was proposed and studied to extract phenolic compounds from spruce wood bark. SVM methodology, in combination with GA, was developed and successfully applied to obtain the working conditions, leading to a maximum polyphenols concentration. The optimal conditions for total phenolic content were obtained using SVMs and GA, which allowed obtaining a polyphenol yield of 58.25 mg GAE g^{-1}, using the following parameter values: extraction temperature of 60 °C, extraction time of 3 minutes and concentration of ethanol of 50%. The accurate results obtained represent the proof that a reliable SVM model was designed and that GA is an adequate solving method for the optimization technique.

Thus, this study, carried out through experiment and simulation, can provide useful information for recovering phenolic compounds from spruce bark, which also indicates that microwave-assisted extraction is a very useful tool for the extraction of important bioactive compounds from plant materials.

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