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Ultrasound-Assisted Extraction of Total Polyphenols from Black Poplar (*Populus nigra)* and Evaluation of Antioxidant Potential

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ABSTRACT
The main objective of this work was to investigate the extraction of total polyphenols from black poplar (BP) (Populus nigra) and their antioxidant activity by 2,2-diphenylpicrylhydrazyl radical scavenging assay. Was evaluated the following variables on extraction step: Temperature, plant-to-solvent ratio, ethanol percentage, ultrasound power, and posteriorly obtained a model to represent total polyphenols content extracted. Total polyphenols reached a maximum value of 103.6 mg gallic acid equivalents. (g of BP^{-1}*) and we concluded that ethanol percentage, plant-to-solvent ratio, and ultrasound power presented significant effect on yield these compounds. On the other hand, temperature had no significant effect indicating energetic relevance and preservation of compounds. Polyphenols-enriched extracts showed antioxidant ranging from 30% up to 90%. P. nigra is an important source of polyphenols suggesting various benefits to human health and use in food products.*

Key words: *Black poplar, Ultrasound-assisted extraction, Total polyphenols, Antioxidant activity.*

1. INTRODUCTION

Polyphenolic compounds are associated with lower rates in coronary heart disease, cancer, and diabetes [1-4] and these compounds act against oxidation of lipids and proteins [5,6] due to the ability to capture and react with free radicals. In particular, compounds derived from plants, such as phenolic acids, flavonoids and tannins possess antioxidant, and anti-inflammatory activities to the prevention of many pathologies, including cardiovascular disease [7,8].

Populus nigra, commonly known as black poplar (BP), is a member of the Salicaceae family and grows in Southern and Central Europe, Central Asia, Siberia, North America, and Brazil. Their leaves are used in traditional medicine to treatment endothelial dysfunction such as inflammations, arthritis, bronchitis, and respiratory tract diseases. A scientific validation of pharmacological actions this plant has been reported by Debbache-Benaida *et al.*, [8], Jerkovic and Mastelic [9], and Nagy *et al.,* [10].

Polyphenols-enriched extracts can be obtained from different techniques and currently, ultrasound-assisted extraction (UAE) is used to overcome drawbacks of conventional extraction, as solvent excess and extraction time [11]. Solid-liquid extraction assisted by ultrasounds leads to intensification of the mass transfer process and increases solvent penetration into the plant. Cavitation cause collapse that promotes cellular disruption together with penetration of solvent into cells through ultrasonic jet [12,13]. In parallel, is desirable to check the potential of extract, proving their antioxidant efficiency against free radicals and perform mathematical modeling of experimental data to optimize, simulate, and control the technological process under study.

In this paper, our objectives were: (i) Study of extraction of total polyphenols from BP (*P. nigra*), investigating the effects of temperature, plant-to-solvent ratio, ethanol percentage, and ultrasound power on extraction yield; (ii) perform mathematical modeling of experimental data; (iii) determine antioxidant activity (AA) by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay.

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2. MATERIALS AND METHODS

2.1. Chemicals

Anhydrous ethanol (Dinamica, 99.5% purity) gallic acid and Folin–Ciocalteu's phenol reagent (Sigma-Aldrich, St. Louis, USA).

2.2. Experimental Design

Samples of BP, obtained in Ibiaçá city, North of Rio Grande do Sul/Brazil (latitude - 28° 03′ 25″; longitude - 51° 51′ 17″; altitude 620 m) were manually collected in September 2012, dried in an air-circulation oven at 303.15 ± 0.5 K and ground in Wiley mill (Tecnal). After this procedure, all material was stored in glass containers and placed in the domestic refrigerator until further use. An experimental design based in Rodrigues and Iemma [14] was employed to study the extraction process of total polyphenols and will be described in section "Results and Discussion, Table 1" together with all results. This procedure included the investigation of temperature, plant-to-solvent ratio, ethanol percentage, and ultrasound power.

2.3. UAE

Extraction assays were performed in test-tube coupled to an ultrasonic bath (Scientz, model SB-5200DTDN) with controlled temperature. Ultrasonic apparatus has a frequency of 40 kHz and maximum output power of 250 W. Ultrasonic transducer is located in lower extremities into the tank (length width height – 300 mm × 240 mm × 150 mm). Basically was developed as follow: Solvent previously prepared with ethanol percentage (Table 1) was added to test-tube and after solution reaching temperature desirable (monitored

Table 1: Experimental design (variables, levels) and maximum content of total polyphenols obtained at 60 min of extraction.

Run	Temperature (K)	Plant-to-solvent ratio ($g.mL^{-1}$)	Ethanol (%)	Ultrasound power (%)	Total polyphenols (mg de GAE. g of plant^{-1})
1	−1	−1	−1	−1	32.16
2	+1	−1	−1	−1	30.69
3	−1	+1	−1	−1	7.56
4	+1	+1	−1	−1	19.14
5	−1	−1	+1	−1	33.72
6	+1	−1	+1	−1	49.97
7	−1	+1	+1	−1	28.80
8	+1	+1	+1	−1	29.47
9	−1	−1	−1	+1	34.54
10	+1	−1	−1	+1	28.41
11	−1	+1	−1	+1	18.25
12	+1	+1	−1	+1	19.89
13	−1	−1	+1	+1	69.70
14	+1	−1	+1	+1	64.79
15	−1	+1	+1	+1	45.52
16	+1	+1	+1	+1	48.67
17	0	0	0	−2	11.51
18	0	0	0	+2	93.19
19	0	0	−2	0	12.04
20	0	0	+2	0	11.56
21	0	−2	0	0	103.60
22	0	+2	0	0	33.28
23	−2	0	0	0	52.82
24	+2	0	0	0	30,02
25	0	0	0	0	52.79
26	0	0	0	0	58.12
27	0	0	0	0	55.70

GAE=Gallic acid equivalents

with a thermometer, Alla, graded 223.15-473.15 K, France), an amount of sample (weighed on analytical balance, Metler/Toledo) was added to system and remained in contact during 60 min. After this time, an aliquot (2 ml) was filtered using a membrane (Millipore, Fluoropore, polytetrafluoroethylene - with a porosity of 0.45 μm) and the resultant solution was used to analyze total polyphenols content (TPC).

2.4. Determination of TPC

TPC was determined by Folin–Ciocalteu reagent, according to Hagerman *et al.*, [15] with some modifications. Aliquots (50 μl) were transferred to test-tubes and the volume completed with 5 ml of distilled water. Was added 0.20 ml Folin–Ciocalteu reagent, 0.5 ml saturated aqueous sodium carbonate solution and then, tubes were vortexed and absorbance measured at 765 nm by spectrophotometer (Shimadzu, UV-1800) after 20 min. TPC was calculated as gallic acid equivalents (GAE) using gallic acid as a standard solution. Results were expressed as mg of total phenolic content (GAE) per gram of dry vegetable mg GAE. (g of BP^{-1}).

2.5. Free Radical Scavenging Activity

Extract (from assay 21) was evaluated according to scavenging activity by DPPH radical scavenger method, adopting procedure described by Brand-Williams *et al.*, [16] with some modifications. Concentrations this extract, ranging of 0.001-0.01g.mL^{-1}, were mixed with 0.06 mM of DPPH methanol solution and absorbance measure at 517 nm (Shimadzu, UV-1800) during 30 min in the dark. Absorbance values were converted to AA% (Equation 1) based on standard curve of DPPH. Results of DPPH assays are expressed as mean of triplicates.

$$\text{Antioxidant activity (\% inhibition)} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right] \times 100 \qquad (1)$$

2.6. Data Analysis

Effects of temperature (303.15-323.15 K), plant-to-solvent ratio (0.02-0.1 g.mL^{-1}), ethanol percentage (10-90%), and ultrasound power (0-100%) on extraction yield were determined by means of experimental design. A central composite rotational design was used to evaluate all effects considering a significance level of 95% ($p<0.05$) (Statistica® 7.0, Statsoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSIONS

Table 1 shows the experimental design (variables and levels) and maximum TPC extracted during 60 min. Can note that in all assays, a high TPC was extracted, reaching a maximum value of 103.6 mg GAE. (g of BP^{-1}) under following conditions: Temperature of 313.15 K, plant-to-solvent ratio of 0.02 g.mL^{-1}, ethanol 50%, and ultrasound power 50%. On the other hand, assay 3 showed low extraction yield, with a maximum value of 7.56 mg GAE. (g of BP^{-1}) at 308.15 K, 0.8 g.mL^{-1}, ethanol 30%, and ultrasound power of 25%.

Results obtained in this work can be considered satisfactory and promising compared to other research found in scientific literature. For example, Debbache-Benaida *et al.*, [8] performed an extraction of *P. nigra* with ethanol (1:6; w:v) at room temperature/24 h and found the value of 51.78 mg catechin equivalent. (g of $\text{extract})^{-1}$. Liu *et al.*, [17] intensified the extraction process of polyphenols from grape seeds with high voltage electrical discharges, concentration by ultrafiltration, and obtained a maximal concentration of 8.3 g GAE (100 of dry $\text{matter})^{-1}$. In comparison with research involving UAE, experimental results can be considered relevant and satisfactory, regarding TPC. Da Porto *et al.*, [13] found values that ranging from 68.47 to 105.81 mg GAE (g of grape $\text{seed})^{-1}$. Pingret *et al.*, [18] concluded that total phenolics content obtained by UAE was 30% higher than those obtained by conventional extraction (555 and 420 mg of catechin equivalent. [100 g of dry $\text{weight}])^{-1}$, respectively.

Results showed in Table 1 were used to estimate effects of each independent variable on TPC extraction process. From these effects, represented in a Pareto Chart (Figure 1a) can be observed that ethanol (%), plant-to-solvent ratio, and ultrasound power had a significant effect linear ($p=0.05$) on extraction step. An increase in plant-to-solvent ratio promotes a decrease in the amount of total polyphenols extracted. On the other hand, in higher ethanol percentage, ultrasonic power, and the synergy of these variables resulted in an increase of TPC extracted. Furthermore, noted that temperature range had no significant effect on the extraction process.

Statistical analysis was performed, and an empirical coded model of TPC was obtained and presented in Equation (2). The coded model was validated by analysis of variance with high correlation coefficient ($R=0.89$) and satisfactory performance of F-test. "R" value showed high significance, allowing use this model to predict extraction process. Therefore, Equation (2) is predictive of TPC in range investigated.

$$TPC = 55.54 - 4.93\ T^2 - 11.14\ C + 1.82\ C^2 + 7.46\ Et - 12.34\ Et^2 + 10.90\ Pot - 2.2\ Pot^2 - 2.08\ T\ Pot + 4.70\ Et\ Pot \qquad (2)$$

Where, TPC is the total polyphenols content after 60 min of extraction, "T" is the coded temperature, "C" is the coded plant-to-solvent ratio, "Et" is the coded ethanol percentage, and "Pot" is the coded ultrasound power.

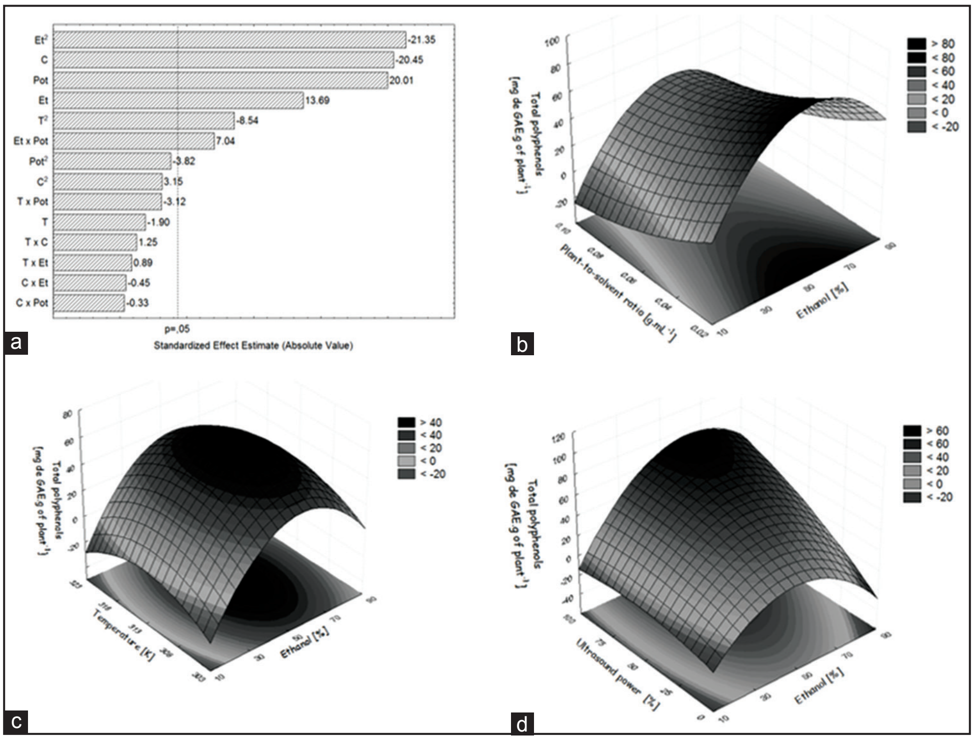

Figure 1: (a) Pareto chart of the standardized effects of independent variables on total phenolics compounds (TPC) extraction, (b) three-dimensional (3D) surface of extraction of total polyphenols from black poplar (BP) as function of plant-to-solvent ratio versus ethanol, (c) 3D surface of extraction of total polyphenols from BP as function of temperature versus ethanol, (d) 3D surface of extraction of total polyphenols from BP as function of ultrasound power versus ethanol.

In sequence, Figure 1b-d shows three-dimensional response surface curves of TPC as a function of plant-to-solvent ratio versus ethanol percentage; temperature versus ethanol percentage; and ultrasound power versus ethanol percentage, respectively. Ethanol percentage (30-70), the lowest solid-to-solvent ratio (0.02 g.mL^{-1}) and extraction at moderate temperature (303.15 K) leads to a high TPC extracted.

These results can be considered important from energy viewpoint and preservation of compounds because the use of low temperature can reduce costs and prevent possible degradation of bioactive principles, maximizing consequently, their antioxidant effects and other benefits desirable. In sequence, Figure 2 shows AA of BP by DPPH method.

From Figure 2 can be observed that values ranged from 30% to 90% (1250 mg.L^{-1} and 10,000 mg.L^{-1}, respectively) and occurred an increase in potential antioxidant directly proportional to concentration until 5000 mg.L^{-1}. On the other hand, was possible to reach a maximum activity (about 90%) using a concentration of 5000 mg.L^{-1} having no necessity to increase their concentration to 10000 mg.L^{-1} to obtain the same value. These values are considered satisfactory when compared with those obtained by Filippi *et al.*, (2015) [19] where authors investigated the AA of

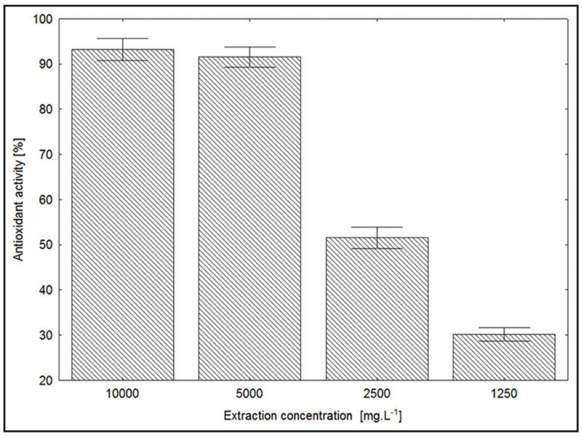

Figure 2: Antioxidant activity of *Populus nigra* at different extracts concentration.

pitanga (*Eugenia uniflora* L.) and found values ranged between 9.18-48.84% for a concentration of 0.001 and 0.01 g.mL^{-1}, respectively.

4. CONCLUSIONS

This research investigated the use of ultrasound-assisted technology to extract TPC from BP (*P. nigra*). Was possible verify the behavior of all variables involved in extraction step and to identify the best conditions by response surface curve and mathematical model. Antioxidant activity of enriched-polyphenols extract reached a value up to 90% for a concentration of 5000 g.L^{-1}. In summary, *P. nigra* is an important source of polyphenols with various benefits. However, complementary studies should be developed to prove no toxicity of extracts in human systems or food products.

5. ACKNOWLEDGMENTS

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***Bibliographical Sketch**

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