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RESEARCH ARTICLE

Biological profiles of *Q. cerris*, *Q. dalechampii*, and *Q. robur* bark extracts: A characterization study

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Objective: The main objective of the present study was to characterize the extracts obtained from the bark of three oak species in order to assess their use in potential cosmetic products.
Methods: The extracts were obtained from the oak barks (periderm and rhytidome) using ultrasound-assisted extraction. The total polyphenolic content was assessed afterward, using the Folin–Ciocâlteu method, while the antioxidant capacity was determined using methods based on the neutralization of the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl radicals. To assess the tyrosinase inhibitory effect, a protocol using L–DOPA as the substrate of the enzyme was employed.
Results: The extracts presented high levels of polyphenolic compounds, with Q. cerris having the highest content. Because of the high concentration of the extracts in polyphenolic compounds, they revealed a great reducing capacity against both DPPH and ABTS radicals, but unfortunately the tyrosinase inhibitory activity of the tested extracts was very weak compared to the positive control.
Conclusions: The extracts may have beneficial effects when used in cosmetic products because of the antioxidant effects, but more studies must be conducted for the determination of the main phytochemical compounds comprised in the extracts and their correlation to the biological effects.

Keywords: *Quercus*, antioxidant, oak, polyphenols, tyrosinase

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Introduction

The *Quercus* genus comprises approximately 400 species of trees (widely known as oaks), being a part of the Fagaceae family. The members of the genus are widely spread especially in Europe, Asia, Northern Africa, and the Americas, representing a big portion of the trees present in the northern hemisphere [1]. Due to this large spread, oak woods very good mechanical properties and durability, it is widely used in carpentry, constructions and furniture manufacturing [2]. Because of these properties oak wood is intensely processed, which results in high quantities of secondary products, such as bark. Moreover, vegetal materials obtained from the species of the *Quercus* genus were used throughout history for the treatment of different pathologies like gastro-intestinal (GI) tract dysfunctions and as antiseptic agents in the treatment of infections [3, 4]. In cosmetology, extracts obtained from the periderm and rhytidome of oak are usually used because of their astringent, antioxidant, and anti-inflammatory effects, being incorporated in cosmetic products like tonners, moisturizing creams or face masks, improving the skin aspect and health [5, 6].

The main compounds from oak barks that offer these effects to the extracts are represented mainly by phenolic compounds such as flavonoids, coumarins, and tannins [7, 8]. Phenolic compounds represent one of the largest and most important groups of secondary plant metabolites, being present in a large number of vegetal species and having numerous beneficial pharmacological properties [9]. These effects are mainly the result of the compounds' antioxidant capacity. There are several proposed mechanisms for this effect, but generally, these phenolic compounds represent chemical entities that can donate electrons to free radicals that occur in the human organism, thus stabilizing the free radical molecule. As a result, the phenolic compound becomes a free radical, but because of the aromatic structure of the molecule, the unpaired electron is delocalized stabilizing the newly formed radical [10, 11].

Taking into account the fact that oak bark represents a by-product of wood processing, the lack of it's valorization and it's content in bioactive compounds (mainly phenolic compounds), this vegetal material represents an important resource for studies trying to assess the exact active compounds present in this vegetal matrix and their biological effects. The aim of this study was to assess the total polyphenolic content, antioxidant activity and the antityrosinase effect of the periderm and rhytidome extracts obtained from 3 oak species, namely *Q. cerris, Q. dalechampii* and *Q. robur*.

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Methods

Chemicals and reagents

The solvent used for the extraction step was ethanol (95%) purchased from Girelli Alcool SRL (Zibido, San Giacomo, Italy). The reagents used for the determination of the total polyphenolic content were purchased: Na_2CO_3*$10H_2O$ from Reactivul SRL (Râmnicu Vâlcea, Romania), Folin–Ciocâlteu reagent from Merck KGaA (Darmstadt, Germany) and gallic acid monohydrate from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and Trolox used for the antioxidant capacity assays were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Lastly, the phosphate-buffered saline (PBS), mushroom tyrosinase (T3824), kojic acid and levodopa (L-DOPA) used for the tyrosinase inhibition assay were all purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Collection of vegetal material

The samples were collected in Deva, Hunedoara County, Romania in May 2021, using the itinerary method. The rhytidome was collected from the tree trunk, at a height of about 1m, while the periderm was collected from the young branches. The chosen specimens were between 15 and 20 years old. The samples were then dried at room temperature, in a dark place. The vegetal material was milled before the extraction using a Pulverisette 15 cutting mill (Fritsch GmbH, Idar-Oberstein, Germany).

Extraction

The milled bark powder was weighed in a beaker (5 g), adding 50 mL of extraction solvent (44% ethanolic solution). The mixture was then sonicated for 15 minutes at an amplitude of 40% using a UP200St sonicator from Hielscher Ultrasonics (Teltow, Germany) [12]. The obtained extracts were filtered under vacuum through filter paper and concentrated afterwards using a rotary evaporator, thus eliminating a great part of the ethanol from the mixture. The concentrated extracts were then frozen and freeze dried using a BK-FD12S freeze dryer (Biobase Biodustry Co., Ltd., Shandong, China). This process resulted in six freeze dried extracts (Table I).

Determination of Total Phenolic Content (TPC)

The polyphenolic content was quantified using a slightly modified version of the Folin–Ciocâlteu method [13]. The extracts were diluted until a concentration of 0.4 μg/mL was reached. 400 μL of the diluted extract was then mixed with 400 μL of Folin–Ciocâlteu reagent and 3200 μL of 5% Na_2CO_3 solution, shaking the test tubes and leaving them in darkness at room temperature for one hour. Two replicates were prepared for each extract, each replicate being measured three times, resulting in six absorbance values. The absorbance was measured at 750 nm with a Specord 200 Plus (Analytik Jena AG, Jena, Germany). The results were then quantified using the results of a nine point calibration curve (range from 0.05 to 0.45 mg gallic acid/ mL). The results were expressed as mg GAE/g dried bark.

Table I. Extracts obtained from different *Quercus* species

Extract	Source
QCP	*Q. cerris periderm*
QCR	*Q. cerris rhytidome*
QDP	*Q. dalechampii periderm*
QDR	*Q. dalechampii rhytidome*
QRP	*Q. robur periderm*
QRR	*Q. robur rhytidome*

Quantification of Antioxidant Capacity

For both antioxidant capacity assays, previously described methods were used [14]. Briefly, serial dilutions of the samples were performed in microplates in the case of both methods (50 μL of sample solution in each well).

In the case of the DPPH method, 200 μL of a 0.1 mM DPPH solution were added in each well. The absorbances of the wells were determined using a Multiskan FC microplate reader (Thermo Fisher Scientific, Waltham, USA). The absorbances were measured at 515 nm, after leaving them at room temperature in darkness for 30 minutes and were compared to a Trolox (1mM solution) positive control. The inhibitory capacity (IC%) was calculated using equation 1. The final results were finally expressed as IC_{50} (representing the concentration of sample which reduced 50% of the DPPH radical):

$$IC\ (\%) = (A_{DPPH} - A_S)/A_{DPPH} \times 100, \quad (1)$$

where A_{DPPH} is the absorbance of the control solution (DPPH solution with solvent) and A_S is the absorbance of the sample.

In the case of the ABTS method, 200 μL of a 10 mM ABTS solution were added to each well. The absorbances were measured at 734 nm, on the same microplate reader, after an incubation time of 10 minutes. The results were compared to the 1 mM Trolox solution. The inhibitory capacity (IC%) was calculated using equation 2. The final results were finally expressed as IC_{50}:

$$IC\ (\%) = (A_{ABTS} - A_S)/A_{ABTS} \times 100, \quad (2)$$

where A_{ABTS} is the absorbance of the control solution (ABTS solution with solvent) and A_S is the absorbance of the sample.

Tyrosinase inhibitory capacity

The experimental protocol used for this assay was previously described [14]. Briefly, serial dilutions of each extract were performed in a microplate, in potassium phosphate buffer. The mushroom tyrosinase solution was then added to each well and the plate was incubated for 5 minutes at 37 °C. L-DOPA was finally added as the substrate of the enzyme. The absorbance of each well was measured at

492nm, using a microplate reader. The final results were expressed as inhibition percentages. Kojic acid was used as a positive control.

Statistical analysis

The means of the TPC values were compared by using a Brown-Forsythe and Welch ANOVA test after determining if the samples were normally distributed by using the Kolmogorov-Smirnov normality test and after testing if there were differences between the standard deviations of the sample sets. The level of significance (α) was set at 0.05 before performing any statistical calculations. Significant differences were considered at p values that were less than the level of significance ($p < \alpha$).

In order to determine the correlation between the TPC and the results of both antioxidant capacity assays, the Pearson coefficient was computed. The Pearson test was used after determining the normality of the result sets using the Kolmogorov-Smirnov test.

Results

Total Phenolic Content (TPC)

The results obtained after the determination of the phenolic content of the six experimental variants revealed major differences between them. The highest contents of phenolics were found in the *Q. cerris* variants, the extracts obtained from the rhytidome being richer in bioactive compounds compared to the periderm variant, but the difference was statistically not significant. The polyphenolic content of the *Q. cerris* extracts was higher when compared to the rest of the variants, but only QCP (368.35 ± 22.03 mg GAE/ g d.w.) was significantly higher as the results obtained for QCR (393.59 ± 70.35 mg GAE/g d.w.) were more unevenly distributed.

The *Q. robur* variants followed the same trend, the rhytidome extract containing a higher level of phenolics compared to the periderm extract, the difference being significant this time (QRP: 227.12 ± 14.94 mg GAE/g d.w. and QRR: 315.59 ± 6.71 mg GAE/g d.w.). Surprisingly, the *Q. dalechampii* extracts have shown a higher concentration of phenolics in the periderm extracts but the difference between the two extracts was not significant (QDP: 265.82 ± 58.23 mg GAE/ g d.w. and QDR: 253.14 ± 2.76 mg GAE/g d.w.). The results are presented in Figure 1.

Antioxidant capacity

The results of the antioxidant capacity assays are represented in Figure 2. For the ABTS assay, the lowest IC_{50} was 0.39 µg/mL in the case of the QCR variant, while QDR had the highest IC_{50} value (1.86 µg/mL). Both *Q. robur* variants presented similar IC_{50} values, having the strongest antioxidant activity after the QCR variant. The Pearson correlation coefficient between the IC_{50} and TPC values was r=-0.4217 indicating a moderately negative correlation.

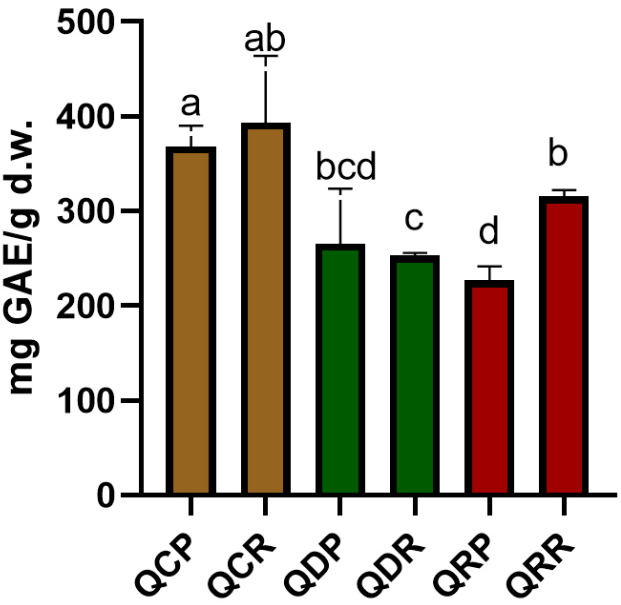

Fig. 1. Total phenolic content of the experimental variants. QCP – extract obtained from *Q. cerris* periderm, QCR – extract obtained from *Q. cerris* rhytidome, QDP – extract obtained from *Q. dalechampii* periderm, QDR – extract obtained from *Q. dalechampii* rhytidome, QRP – extract obtained from *Q. robur* periderm, QRR – extract obtained from *Q. robur* rhytidome; the results are represented as mean ± SD; different letters represent significant differences.

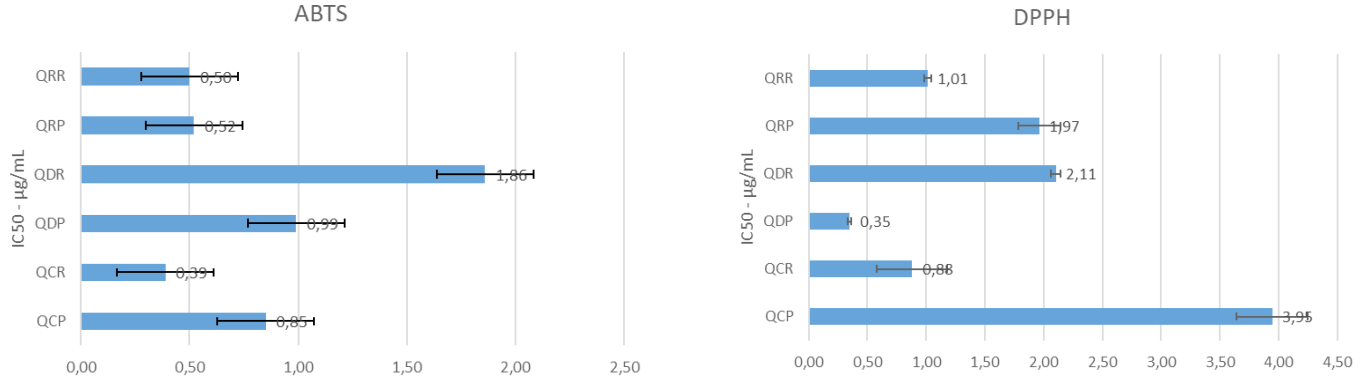

Fig. 2. Extracts' antioxidant capacity for both antioxidant assays (ABTS and DPPH). QCP – extract obtained from Q. cerris periderm, QCR – extract obtained from Q. cerris rhytidome, QDP – extract obtained from Q. dalechampii periderm, QDR – extract obtained from Q. dalechampii rhytidome, QRP – extract obtained from Q. robur periderm, QRR – extract obtained from Q. robur rhytidome.

Surprisingly, for the DPPH assay the lowest IC_{50} value was determined for the QDP variant and the highest for the QCP variant, while the QCP variant had a significantly higher TPC compared to QDP. Because of these results, the Pearson correlation coefficient showed a very weak positive correlation between the IC_{50} and TPC values (r=0.1595).

Tyrosinase inhibitory activity

The results regarding the inhibition capacity of the tyrosinase are presented in Figure 3. It can be observed that the experimental variants have a very similar behavior regarding the trend of inhibition. All the tested extracts inhibit the enzyme at very similar concentration levels.

Even though the extracts presented similar trends the overall inhibitory capacity of each extract is weak when compared to the kojic acid positive control (IC_{50} = 5 µg/ml), as revealed by the IC_{50} values illustrated in Figure 4.

Discussions

In order to validate our results and reach certain conclusions regarding the quality and bioactive compounds quantification of our tested extracts, our data were compared to data found in various studies which were conducted with similar tests. In a previous study conducted on *Q. cerris* ethanolic rhytidome extracts a TPC of 403.73 ± 7.35 mg GAE/g d.w. was obtained, a result similar to our findings [15]. Although a bit higher, the difference between these results might come from the different extraction method (microwave-assisted extraction) used or different ethanol concentration utilized for the preparation of the extraction solvent. Previously obtained data focused on the efficiency of different extraction methods used on hard vegetal matrices like the bark, highlighted a higher efficiency of the microwave-assisted extraction when compared to the regular ultrasound-assisted extraction [16, 17]. Similarly, for *Q. robur* rhytidome extracts a level of 347.74 ± 8.66 mg GAE/g d.w. was highlighted previously for extracts obtained via microwave-assisted extraction indicating once again a higher TPC when compared to our extracts. However, the same study showed a much lower TPC for the extracts obtained through ultrasound-assisted extraction (240.99 ± 1.49 mg GAE/ g d.w.), which is a smaller content when compared to our result [18]. The comparison also shows the higher efficiency of sonication with a probe compared to regular ultrasound-assisted extraction (with an ultrasound bath). In contrast, a higher TPC was obtained for *Q. dalechampii* when microwave-assisted extraction and regular ultrasound-assisted extraction were deployed, in comparison to our results [14].

Regarding the antioxidant capacity of the tested extracts, our acquired data suggests lower IC_{50} values when compared to previous published data in which similar extracts were tested with the same antioxidant assays (ABTS and DPPH) [19–21]. Moreover, the different results obtained for the two antioxidant assays may be explained by the different responses of each specific radical to the antioxidant molecules and also by the different phytochemical profiles of the extracts [22]. Unfortunately, this hypothesis cannot be proven under current circumstances, as no data was acquired regarding the detailed phytochemical profile of the tested extracts.

The tyrosinase inhibitory potential of oak bark extracts was highlighted previously in certain research papers. Some examples would be the inhibitory effect of *Q. coccifera* [23], *Q. mongolica* [24] and *Q. rubra* [25] on tyrosinase. The IC_{50} values presented in the articles above are lower compared to our findings, however they are still higher when compared to the positive controls. This indicates that the extracts might contain specific compounds that can inhibit the enzyme, such as resveratrol (because of its resorcinol group in its structure) [26], but not in a high enough concentration in order to exert a potent effect.

Conclusion

Quercus species are well known as a source of bioactive compounds, mainly polyphenols, with multiple applications in cosmetic and pharmaceutical products, while lit-

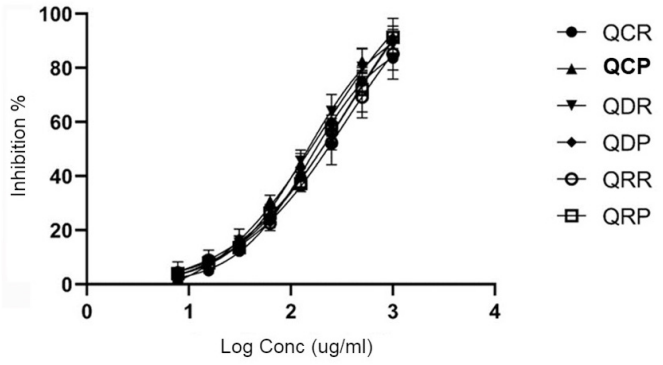

Fig. 3. Tyrosinase inhibition curves of the tested extracts. QCP – extract obtained from *Q. cerris* periderm, QCR – extract obtained from *Q. cerris* rhytidome, QDP – extract obtained from *Q. dalechampii* periderm, QDR – extract obtained from *Q. dalechampii* rhytidome, QRP – extract obtained from *Q. robur* periderm, QRR – extract obtained from *Q. robur* rhytidome.

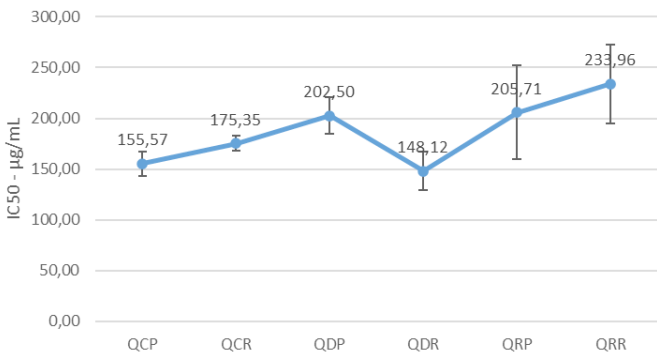

Fig. 4. IC_{50} values of the tested extracts against tyrosinase. QCP – extract obtained from *Q. cerris* periderm, QCR – extract obtained from *Q. cerris* rhytidome, QDP – extract obtained from *Q. dalechampii* periderm, QDR – extract obtained from *Q. dalechampii* rhytidome, QRP – extract obtained from *Q. robur* periderm, QRR – extract obtained from *Q. robur* rhytidome.

erature supports the use of oak bark as an alternative polyphenols source. Our results suggested that polyphenolic compounds are distributed less in the periderm, resulting in a lower antioxidant capacity of the extracts obtained from this vegetal matrix. Also, the results of the antioxidant assays revealed different variation trends depending on the free radical. Moreover, the inhibitory capacity of the tyrosinase did not vary significantly with the polyphenolic content, but overall, the enzymatic inhibition was weak. More studies should be performed regarding the specific phytochemical profile of the extracts for a better understanding of their biological activities.

Authors' contribution

AN (Investigation; Methodology; Writing – original draft)
N-A.C. (Formal analysis; Investigation; Visualization;)
M.B (Formal analysis; Investigation; Visualization;)
SS (Formal analysis; Investigation; Methodology; Writing – original draft)
O.F. (Formal analysis; Investigation)
CT (Conceptualization; Formal analysis; Investigation; Project administration; Resources; Visualization; Writing – review & editing)

Conflict of interest

None to declare.

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