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Published at http://ijate.net/index.php/ijsm http://dergipark.org.tr/ijsm **Research Article**

Quantitative analysis of Polydatin in a Turkish oak: *Quercus coccifera* L. with HPLC-DAD

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Abstract: In this study, a new, simple, rapid and sensitive HPLC-DAD method was used for analysing polydatin contents of *Quercus coccifera* (Fagaceae) woody parts extracted with methanol and water. Our results showed that methanol and water extracts of *Q. coccifera* had high polydatin contents: 14.898±0.147 and 5.574±0.112 mg/g dry extracts, respectively. This is the first developed analytical method for qualitative and quantitative analysis of polydatin in *Quercus* L. species.

ARTICLE HISTORY

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KEYWORDS

Quercus coccifera L., Oak, Fagaceae, HPLC-DAD, Polydatin

1. INTRODUCTION

Plants have been a very important source of traditional medicine for years and continuously provided new therapies for humankind. Great efforts have been made to identify and quantify natural active ingredients from plants using various techniques. Resveratrol is now gaining much attention because of its antiaging, anticancer and antioxidant effects. Polydatin (PL), also named piceid, is a resveratrol glucoside (*trans*-resveratrol 3-*O*-glucoside) (Figure 1) isolated from *Polygonum cuspidatum* and other genera such as *Rosa*, *Rumex*, *Picea*, *Arachis*, *Malus* and *Quercus*. It is also detected in hop cones, red wines, hop pellets, cocoa-containing products, chocolate products and many daily diet components [1,2]. Enzymatic, microbiologic or chemical methods are used for transforming PL to resveratrol. *cis*-Resveratrol and *cis*-polydatin are typically found at lower concentrations and are often less biologically active than their *trans* forms. When compared to resveratrol, PL demonstrates better antioxidant activity and higher bioavailability. Therefore, PL stands out as an attractive compound to conduct more research on [3-5]. It has various biological activities such as antiinflammatory, antioxidant, antishock, anticancer, antimicrobial, neuroprotective, lung protective and hepatoprotective effects [1]. Chinese FDA approved PL for multiple phase II clinical trials mainly for antishock

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applications [6]. In connection with promising results from bioactivity studies, interest in this compound increased.

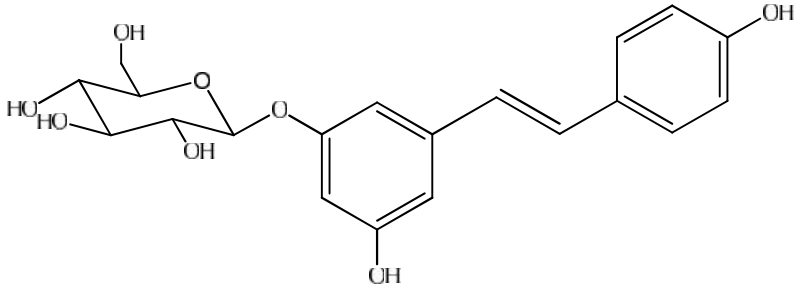

Figure 1. Chemical structure of Polydatin

The *Quercus* L. (Fagaceae) genus has 23 species naturally distributed in Turkey, of which four are endemic [7]. Intense research has been focused on *Quercus* sp. since it is used not only by humans and animals as food but also for its contribution to different industries including winemaking and wood. Barrels made from oak are usually used for wine-maturation process. Oak is used for colouring wood and protection against fungal decay. In Turkish traditional medicine, *Quercus* plants have been used as antiseptic, antidiarrheal, hemostatic, wound healing, and stomachic agent as well as remedy for poisoning from alkaloids, copper, lead and heavy metal salts [8,9]. So far, flavonoids, tannins, triterpenes, ionones, phenols, lignans and catechin derivatives were isolated from *Q. coccifera* L., which is native to the Mediterranean region of Anatolia and locally called as “kermes mesesi and pinar”. It is used for the treatment of diabetes and diarrhea. Furthermore, the decoction of this plant is used for burns is used for burns [9-11]. [9-11]. Previously, we also reported the isolation and structure elucidation of phytochemicals such as an ionone derivative, polydatin, lignans and a catechin derivative from the methanolic extract of *Q. coccifera.* Polydatin was one of the major compound in this extract and it was found for the first time in *Quercus* L. genus [11]. The aim of the current study is to determine the PL content of the *Q. coccifera* woody parts, which have already been identified as a new source of PL.

2. MATERIAL and METHODS

2.1. Chemicals

HPLC-grade acetonitrile was purchased from Merck Millipore, Germany. HPLC grade water was obtained from Milli-Q water purification system (18.2 MΩ/cm, Millipore), and analytical grade phosphoric acid was purchased from Merck, Germany. Polydatin (PL), which was used as the standard compound, was purchased from Sigma (15721)

2.2. Plant Material

Quercus coccifera L. (Fagaceae) was collected from Sertavul-Akçeşme between Mut and Konya (Turkey), near roadway at 1600 m in August 2008. It was identified by Prof. Zeki Aytaç (Department of Biology, Faculty of Sciences, Gazi University). A voucher specimen is deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey (HUEF 10003).

2.3. Extraction

Air dried and powdered woody parts (50 g) of *Q. coccifera* were extracted in two different ways. In the first method, same with the traditional usage, plant material was heated in distilled water (2 x 500 mL) at 100 ˚C for 3 hours to prepare a decoction and in the second one, it was extracted in MeOH (2 x 500 mL) at 45 ˚C for 3 hours under reflux. Both of the extracts were

dried in vacuo and lyophilized (Virtis-6KBTES) to obtain the water extract (4 g, 8%) and MeOH extract (3.58 g, 7.2%). Lyophilized extracts were kept at -20 ˚C.

2.4. Equipment and Chromatographic Conditions

The high performance liquid chromatography was performed using Dionex P680 HPLC system with photo-diode array detector (DAD). Samples were injected into the column filled with the stationary C18 (25 cm x 4.6 mm, 5 µm particles, I.D, ACE) with a pre-column (10x4 mm) and a Dionex ASI-100 autosampler at 30 °C. The data were acquired and processed by using Chromoleon 7.2, Thermo Fischer Scientific software. All the calculations concerning the quantitative analysis were performed with external standardization method by measuring the peak areas. Flow rate was set at 1 mL/min and column temperature was set to 30 °C. All the solvents were filtered through a 0.45 µm membrane filter before use and de-gassed in an ultrasonic bath. The injection volume was 20 µL. The mobile phase was a mixture of H_3PO_4 in pH:2.4 buffer system (solution A) and acetonitrile (solution B) with gradient elution. The composition of the gradient was (A:B), 95:5 at 0 min, 90:10 at 5 min, 80:20 at 10 min, 75:25 at 20 min, 70:30 at 30 min and 0:100 at 40 min. The chromatograms were recorded from 200 to 400 nm. Quantification was performed by measuring the areas under curve for PL in both of the extracts at 307 nm using a photo-diode array detector. The chromatographic run time was 40 min.

2.5. Method Development

Various gradient and isocratic methods were tested to detect PL using different literature data [16-20]. Isocratic 17% acetonitrile in 0.5% water formic acid [18] did not give baseline separation but peak splitting of compounds. The HPLC conditions were similar to those described by Lamuela-Ravento´s et al. [16] and Romero-Pérez et al. [17], however, a mixture of H_3PO_4 in pH:2.4 buffer system (solution A) and acetonitrile (solution B) with gradient elution was tried as mobile phase: 0 min, 95.0% A, 5.0% B; 5 min, 90.0% A, 10.0% B; 10 min, 80.0% A, 20.0% B; 20 min, 70.0% A, 30.0% B; 25 min, 60.0% A, 40.0% B; 35 min, 100% B. At the end of all trials, we decided to use our new method given in the HPLC conditions part above.

Sample Preparation: 5 mg water and methanol extracts were weighed and dissolved in 1 mL mobile phase.

Standard Stock Solution: 1 mg PL was dissolved in 1 mL mobile phase.

2.6. Method Validation

Linearity, accuracy and precision of the analysis were determined by using the ICH guidelines [21].

2.6.1. Linearity

An external standard method was utilized to construct the calibration curve. Six different concentrations of PL (1-100 µg/mL, *n*=6) were prepared in mobile phase. 20 µL of each standard solution was injected into the system for 6 times, and then the peak areas obtained from the injections were plotted against the concentrations to establish the calibration graph (Table 1).

2.6.2. Precision

The precision was evaluated by using intra-day and inter-day results. Standard PL was injected to the column at three different concentrations (25-50-100 µg/mL, *n*=6) on the same day for determining the intra-day precision. The same procedure was applied on two different days for inter-day precision.

2.6.3. Limits of Detection (LOD) and Limits of Quantification (LOQ)

The LOD and LOQ values were determined by the signal-to-noise (S/N) method, where an S/N ratio of 3 was used for LOD and 10 for LOQ. LOD and LOQ were experimentally verified by the six injections of reference compound at three different concentrations (25-50-100 µg/mL) [16].

2.6.4. Accuracy

Accuracy was evaluated by adding three increasing concentrations of standard PL (25-50-100 µg/mL) to both water and methanol extracts. Nonspiked extract samples (blanks) were used for calculating the percentage recovery at each concentration.

3. RESULTS and DISCUSSION

In this study, methanol and water extracts of the woody parts of *Quercus coccifera* L. (Fagaceae) which is used as water decoction in folk medicine, are investigated for PL content. This is the first report of HPLC-DAD analytical method developed for quantitative analyse of PL in *Quercus* L. species. This study reports the development of a new RP-HPLC method for the determination of PL in *Q. coccifera*. In conclusion, the quantitative evaluation of PL in *Q. coccifera* is improved with our simple, low-cost, sensitive HPLC method.

Polydatin demonstrated a good linear response (R^2>0.9987), and low LOD (6.8 µg/mL) and LOQ (20.8 µg/mL) values for the analyses within the linear range at 1-100 µg/mL concentration (Table 1). Retention time for PL in these conditions was determined as 16.5 min for HPLC-DAD chromatograms of methanol extract and water extract as given in Figures 2-5. The results of the precision analysis which were found about 1% for both intraday and interday indicated that the good reproducibility of our method (Table 2). The percentage of the mean recoveries of the extracts were in the range of 98.59-103.70% (water extract) and 100.87-102.36% (methanol extract) as seen in Table 3. The identification of PL in the extracts was based on the retention time and the comparison of UV spectra (Figure 6) with those of authentic standard. This method can be used for quantifying this compound in plant extracts and the products. Our results demonstrated that methanol and water extracts of *Q. coccifera* had high PL content: 14.898±0.147 and 5.574±0.112 mg/g (in dry extract), respectively. Compounds in water extract may have been hydrolyzed by this preparation method during boiling with water for 3 hours as used in folk medicine. The preparation method can explain the low amount of the compound (PL) in the water extract. Our results are the first records on the quantity of PL in *Quercus* L. genus. A number of studies reported determination of PL in different genus and food and/or products. For instance, in a previous study, the level of PL was highest (7.14 µg/g) in the cocoa powders [12].

Polydatin is a natural precursor of resveratrol, well known as an antioxidant. According to Hollman et al. [13], Paganga and Rice-Evans [14] absorption of some phenols from diet is enhanced by conjugation with glucose. Furthermore, PL content of *Quercus* is probably responsible for the high radical scavenging activities of *Q. coccifera* [15]. Due to its high PL content, *Q. coccifera* can be a potentially good source of antioxidant effect for food, pharmaceutical and cosmetic industries.

Table 1. Calibration parameters for the detection of polydatin

Linear equation	R^2	Linear range (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)
y=2.593x + 4.8678	0.9987	1-100	6.8	20.8

Table 2. Precision values of polydatin

Concentration (µg/mL)	*Intra-day precision (RSD, %)**	*Inter-day precision (RSD, %)**
25	0.225	0.179
50	0.019	0.248
100	0.909	1.222

*RSD %, percentage of relative standard deviation (*n*=6)

Table 3. Accuracy of the HPLC method for determination of PL in both *Q. coccifera* water and methanol extracts

Extract	*Initial content (µg/mL)*	*Recovery after 25 µg/mL added (%)*	*Recovery after 50 µg/mL added (%)*	*Recovery after 100 µg/mL added (%)*
Water	31.22	99.46	103.70	98.59
Methanol	95.35	100.87	102.00	102.36

4. CONCLUSION

Besides promising results obtained from bioactivity tests and clinical trials, being an option to overcome the bioavailabity problem of resveratrol, it is important to find new sources and quantification methods for polydatin. The novelty of the present study was to apply of HPLC techniques to find its quantification in a Turkish oak: *Quercus coccifera* L. We developed a rapid, simple, sensitive HPLC method for determination of polydatin.

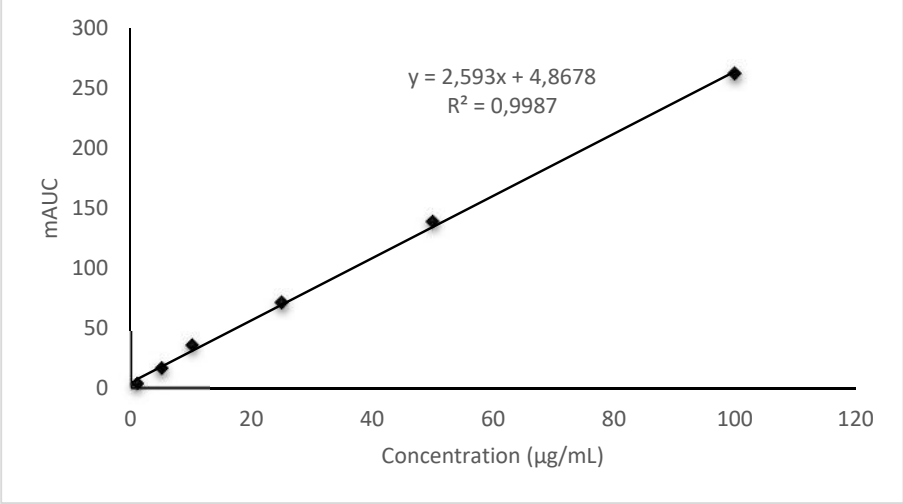

Figure 2. Calibration curve for polydatin

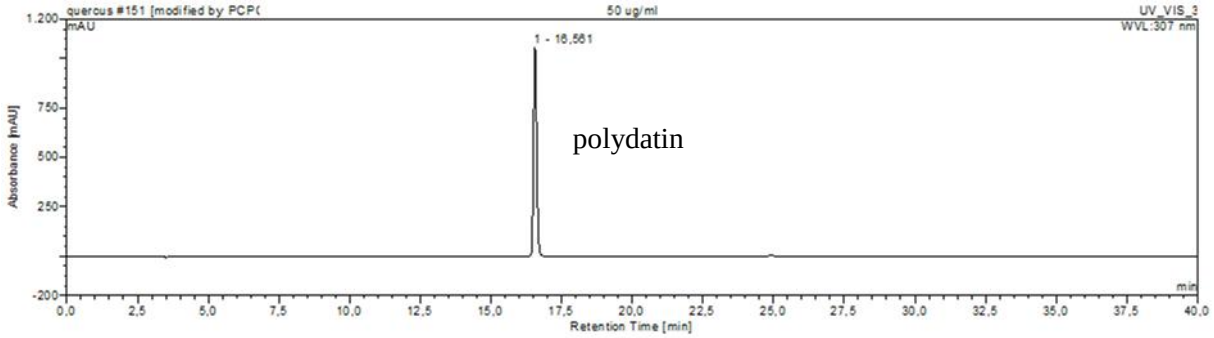

Figure 3. HPLC chromatogram of polydatin

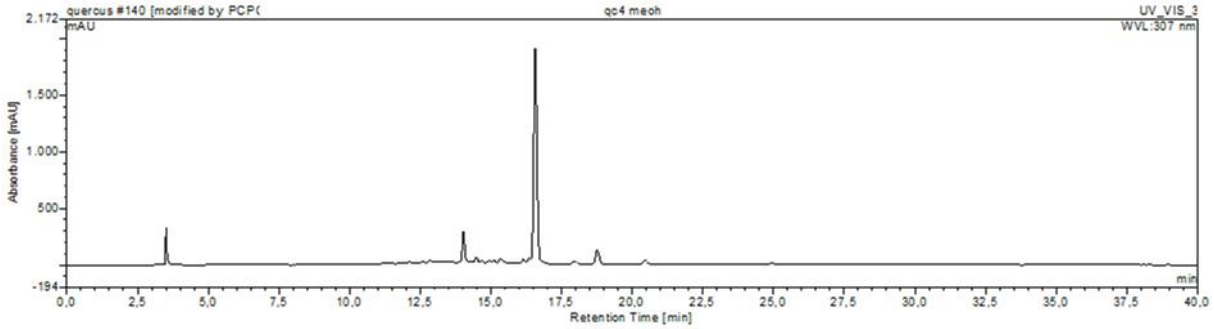

Figure 4. HPLC chromatogram of *Q. coccifera* MeOH extract

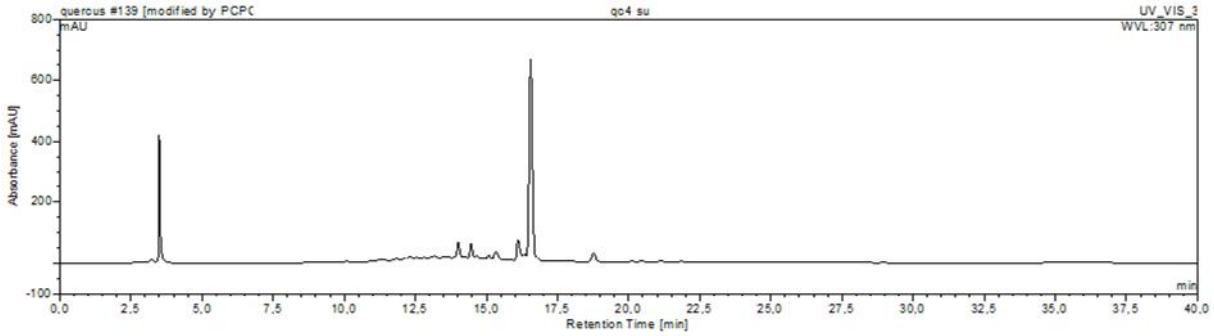

Figure 5. HPLC chromatogram of *Q. coccifera* water extract

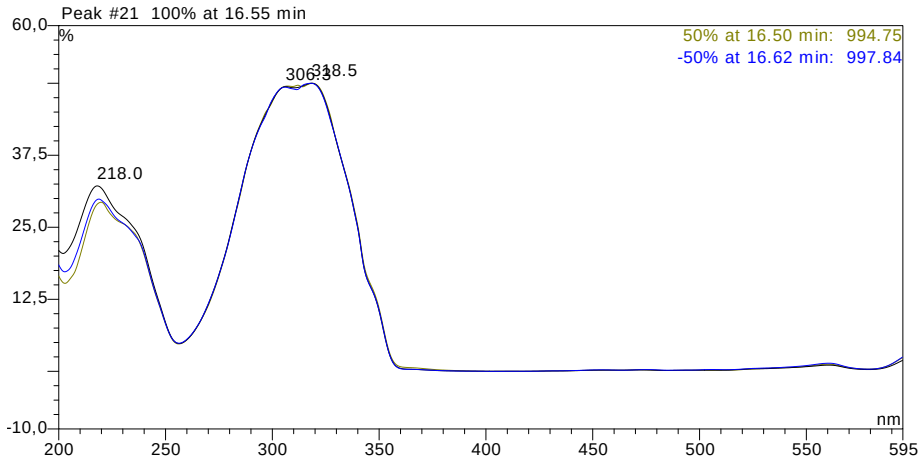

Figure 6. UV spectrum of polydatin in both *Q. coccifera* MeOH and water extracts

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