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RESEARCH ARTICLE

Quantitative Determination of Tannic Acid in Quercus Species by High Performance Liquid Chromatography

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SUMMARY

This study aims to the quantitative analysis of tannic acid in different extracts of Quercus species growing in Turkey. For this purpose, the quantitative analysis of tannic acid was performed in two oak galls (Q.infectoria subsp. infectoria and subsp. boissieri) growing in Turkey by using of High Performance Liquid Chromatography (HPLC) method. Four different solvents were used for extraction procedure. So, tannic acid contents were determined in 96% ethanolic extracts (30.852-81.012 mg/g), 80% ethanolic extracts (43.898-127.683 mg/g), 70% acetone extracts (3.064-67.200 mg/g) and extracts with mixture of diethylether:ethanol:water (0.016-0.112 mg/g) for Quercus infectoria subsp. boissieri and Q. infectoria subsp.infectoria galls, respectively. The most abundant tannic acid has been reported as 127.683 mg/g in the 80% ethanolic extract of Quercus infectoria subsp. infectoria. The limits of detection and quantification were measured as 1.5 ppm and 4.95 ppm, respectively. This is the first study on the quantitative determination of tannic acid for two subspecies of Quercus infectoria (subsp. infectoria and subsp. boissieri) growing in Turkey in literature.

Key Words: *Tannic acid, HPLC, Quercus infectoria subsp. boissieri, Quercus infectoria subsp. infectoria, oak, tannin.*

Quercus Türleri İçinde Tannik Asit'in Yüksek Basınçlı Sıvı Kromatografisi ile Kantitatif Olarak Saptanması

ÖZ

Bu çalışma, Türkiye'de yetişen Quercus türlerine ait farklı ekstrelerdeki tannik asit miktarlarının kantitatif analizini amaçlamaktadır. Bu sebeple, Türkiye'de yetişen 2 mazıdaki (Q.infectoria subsp. infectoria and subsp. boissieri) tannik asit miktarları yüksek performanslı sıvı kromatografisi (HPLC) yöntemi kullanılarak belirlenmiştir. Ekstraksiyon yöntemi için dört farklı çözücü kullanılmıştır. Quercus infectoria subsp boissieri ve Q. infectoria subsp. infectoria için tannik asit içerikleri %96'lık etanollü ekstrede (30,852-81,012 mg/g), %80'lik etanollü ekstrede (43,898-127,683 mg/g), %70 asetonlu ekstrede (3,064-67,200 mg/g) ve dietileter:etanol:sudan ibaret ekstrede (0,016-0,112 mg/g) olarak belirlenmiştir. Tannik asit en yüksek miktarda Quercus infectoria subsp. infectoria'nın %80 etanollü ekstresinde 127,683 mg/g olarak rapor edilmiştir. Tannik asit için saptama ve kantifikasyon sınırı değerleri sırasıyla 1,5 ppm and 4,95 ppm olarak ölçülmüştür. Bu çalışma, Türkiye'de yetişen Quercus infectoria'nın 2 alttüründeki (subsp. infectoria and subsp. boissieri) tannik asitin kantitatif belirlenmesine yönelik literatürdeki ilk çalışmadır.

Anahtar Kelimeler: *Tannik asit, HPLC, Quercus infectoria subsp. boissieri, Quercus infectoria subsp. infectoria, mazı, tanen.*

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INTRODUCTION

Some galls of *Quercus* species (oaks, especially *Q. infectoria*), leaves or nutgalls of *Rhus* species (sumacs, *R. coriaria*, *R. glabra*, *R.thyp*ia, *R.semialata*), galls on *the R.chinensis* (Galla chinensis) and seed pods of Tara (*Caesalpinia spinosa*) contain tannic acid. This hydrolyzable tannin (as gallotannin) and some galls are used for similar purposes i.e. burn injuries, diarrhea, food additive, wound, astringent, styptic and antidote for poisoning in some medicines (Ghosh, 2015; Evans, 2009; Buckingham, 1994; Mueller-Harvey, 2001; Gruenwald *et al*., 2000; Arapitsas, 2012). Also, galls are used for obtaining of tanning and manufacturing of ink and dye. The galls of *Quercus infectoria* (mazı) were traditionally used for dye by burning of them and diarrhea with infusion in İstanbul, Eskişehir and Gaziantep (Başer *et al*., 1986). Fruits of *Q.infectoria* subsp. *boissieri* has been eaten by cooking in East Anatolia (Kızıl *et al*., 2014). Galls of *Q.infectoria* subsp. *infectoria* (mazı meşesi) used for diarrhea and astringent in Çankırı (Tuttu, 2014). Oak galls and sumac leaves contain tannins or tannic acid are used in food, paint and leather industry (Evans, 2009; Shirmohammadi *et al*., 2018; Falcao *et al*., 2018).

Tannic acid has been reported as "Acidum tannicum (Tanin)" , "Tannic acid" or "Tannic acid (Tanninum)" in Turkish Codex, United States, European and Turkish Pharmacopoeias respectively (Türk Kodeksi, 1948; United States Pharmacopoeia, 2007;European Pharmacopoeia, 2008; Türk Farmakopesi, 2016; Wiesneth & Jürgenliemk, 2017). Also, the sources of commercial tannic acid consist of mixture of polygalloyl-quinic or polygaloyl-glucose acid esters (Makkar & Becker, 1993). Some types of oak galls (Aleppo, Chinese or Turkish galls) contain the tannin found as gallotannic acid (or Tannic acid in British or European Pharmacopoeias) between 50-80% while English oak galls contain the tannin maksimum 20 %. Some phenolic acids (gallic, ellagic and syringic acids), ellagitannins (vescalagin, pterocarinin, castalagin, grandinin), sugars (starch), methyl oleanolate, methy betulate, condensed tannins (derivatives of catechin, gallocatechin and epicatechin) and calcium oxalate have been found in some *Quercus* extracts besides tannic acid (as pentadigalloylglucose complex) (Evans, 2009; Tuttu, 2014,; Wiesneth & Jürgenliemk, 2017; Önal *et al*., 2005; Ikram & Nowshad, 1977; Nonaka *et al*., 1990; Zhang *et al*., 2015; Şöhretoğlu & Sakar, 2004).

Quercus genus belongs to the Fagaceae family and comprises many species in Europe, Asia and America. It has more than 18 species with 8 subspecies and 2 varieties in Anatolia, Turkey. *Quercus* species are semi-evergreen small trees or shrubs. It's very important genus for its economic value besides industrial uses. Also, the leaves are semigreen and narrowly oblong to ovate; margins are crenate or serrate or crenate-serrate to entire; petioles between 1-5 mm or (3-)10-25 mm in *Q. infectoria* subsp. *infectoria* and subsp. *boissieri*, respectively (Davis, 1982; Güner, 2012; Uslu *et al*., 2011). It has been reported that some *Quercus* species including *Q. infectoria* and also tannic acid possess astringent, antioxidant, antidiabetic, hepatoprotective, antiviral, anticarcinogenic, anti-inflammatory, anticancer, antifungal and analgesic activities (Ghosh, 2015; Yıldırım & Kutlu, 2015; de Sousa Leal *et al*., 2015; Karakurt & Adalı, 2011; Chung *et al*., 1998; Liu *et al*., 2015; Naim *et al*., 2017; Vanga *et al*., 2017; Genç *et al*., 2012). Also, it has been shown gastroprotective effects of aqueous extract of root barks from *Q. ilex* or ethanolic extract of fruits from *Q. coccifera* and *Q. aegilops* besides anticandidal effect of aqueous extract obtaining from galls of *Q. infectoria* (Shrestha *et al*., 2014; Baharuddin *et al*., 2015). Phenolic acids (gallic, vanillic, syringic and ellagic acids) and flavon glycosides (quercetin, isorhamnetin and kaempferol with their glucosides, etc.), with hydrolyzable tannins have been extensively determined in different parts of the *Quercus* species (acorn, bark, heartwood, leaf, cork, husk of root or root) besides their nutgalls by HPLC, LC-MS (HPLC-ESI-MS, LC-MS/MS) or NMR (FT-NMR, ^{1}H-NMR, ^{13}C-NMR, etc) methods (Önal *et al*., 2005; Nonaka *et al*., 1990; Zhang *et al*., 2015; Garcia-Villalba *et al*., 2017; Karaoğul *et al*., 2016; EMA, 2010).

Firstly; investigation of four commercial tannic acids prepared from gallnuts of *Q. infectoria*, pods of *Caesalpinia spinosa*, leaves of *Rhus cotynus or R. coriaria* and gallnuts of *R. semialata* by Normal and Reversed Phase HPLC analysis. Even they detected percentages of tannic acid besides impurity of gallic acid of these commercial tannic acids (Verzele & Delahaye, 1983). But, no record has been found in extracts of *Quercus infectoria* subsp. *boissieri* and *Q.infectoria* subsp. *infectoria* growing in Turkey and their contents of tannic acid in literature by HPLC or LC-MS methods. Therefore, this study is aimed to evaluate the tannic acid contents in different extracts of *Q.infectoria* subsp. *infectoria* and *Q.infectoria* subsp. *boissieri* galls by HPLC analysis.

MATERIALS AND METHODS

Plant materials

Quercus infectoria subsp. *infectoria* and *Q.infectoria* subsp. *boissieri* were collected from Tokat (No. 1 and 4, grape galls-apple galls) and Batman (No. 2

and 3), respectively during growth period November, 2018. They were deposited in the Herbarium of Anadolu Univesity, Faculty of Pharmacy (ESSE 36, ESSE 37) as given in Figure 1. *Quercus infectoria* subsp. *boissieri* and subsp. *infectoria samples* were analyzed by pulverization.

Figure 1. Plant samples of *Quercus* species *(*grapegalls-apple galls *of Quercus infectoria* subsp. *infectoria* No: 1-3) and *Q.infectoria* subsp. *boissieri* No.4).

HPLC analysis

HPLC analyses were carried out using Shimadzu Prominence model chromatography (Shimadzu Corporation, Tokyo, JAPAN). This system coupled with a 20A CBM (HPLC System Controller), a diode array detector (SPD-M20A), a SIL 20ACHT automatic sampler, a CTO10ASVp column oven and a LC20 AT pump. The diode-array detector was set at 270 nm and peak areas were integrated automatically by Shimadzu software. The chromatograms were plotted and processed with this sofware. The separation was achieved on a Zorbax Eclipse XDB-C18 column (250 x 4.6 mm i.d; 5 ***µm;*** Agilent Technologies, USA) at 25°C. The mobile phase consists of water 3% formic acid (A) versus (B) methanol. The gradient elution were applied at a flow rate of 0.8 mL/min. The elution profile was: 95% A/5% B for 3 min, 80%A/20%B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, and 100% B in 10 min until the end of the run. Samples were dissolved in methanol, and 100 µL of this solution was injected into the column (Caponio *et al*., 1999).

Chemicals and reagents

Commercial tannic acid was purchased from Sigma-Aldrich (403040, ACS, Steinheim, Germany). Methanol (HPLC grade), acetone (GC grade), ethanol (HPLC grade), diethylether (ACS grade) and formic acid (analytical grade) were obtained from Merck (Germany) and Sigma-Aldrich (Steinheim, Germany), respectively.

Calibration standard

Standard was prepared as stock solutions in methanol (10 mg/mL).

Extraction procedure

In the analysis of samples at HPLC, different solvents were used for extraction.

Method 1: The first extraction procedure as follows; plant samples (2 gram) were dissolved in 96% ethanol. After homogenization, they have been stored at 45°C for one night and then centrifuged at 4000 rpm. Supernatant was evaporated in rotavapor (Büchi, R-300, Switzerland) (Kiselev *et al*., 2007).

Method 2: Maceration has been applied the samples (10 gram) by shaking for 8 hours at room temperature with 200 ml methanol (80%). These samples were filtered and concentrated under vacuum by rotary evaporator (Gangwal, 2013).

Method 3: Maceration has been applied the samples (10 gram) by shaking for 8 hours at room temperature with 200 ml acetone (70%). These samples were filtered and concentrated under vacuum by rotary evaporator (Elgailani & Ishak, 2016).

Method 4: Maceration has been applied the samples (10 gram) by shaking for 8 hours at room temperature with 200 ml diethylether:ethanol:water mixture (25:3:1). These samples were filtered and concentrated under vacuum by rotary evaporator (Haque *et al*., 2016).

RESULTS AND DISCUSSION

Our study performed to the quantitative determination of tannic acid in nutgalls of two *Quercus infectoria* subsp. *boissieri* and subsp. *infectoria* collecting from Tokat and Batman Provinces in Turkey. Four different solvents [ethanol, methanol, acetone or (diethylether:ethanol:water mixture) (25:3:1)] have been applied for plant materials. The calibration graph was plotted using peak area values corresponding to tannic acid concentration (x). The limit of detection (LOD), and the limit of quantification (LOQ) are important parameters for the method. A signal-to-noise ratio of 3: 1 is generally considered to be an appropriate ratio for LOD evaluation. A typical signal-to-noise ratio for a LOQ is 10: 1.

The calibration curve, linear regression, LOD and LOQ values for the method are shown in Table 1.

Table 1. Analytical performance of tannic acid analyses in the studied matrices

Compound	Y	R^2	λ	Rt	LOD (ppm)	LOQ (ppm)
Tannic acid	5455.5 + 34611X	0.999	270	65.90	1.50	4.95

Y: regression equation; R^2: correlation coefficient; λ: wavelength; Rt: retention time; LOD: limit of detection; LOQ: limit of quantification

The results of the tannic acid using all Methods (1-4) are given in Table 2.

Table 2. Quantities of tannic acid for different extracts of two *Quercus* species (mg/g)

Sample numbers* with extraction methods	**Tannic acid quantity (mg/g)**	**Relative standart deviation (RSD,%)**
E: Extraction with 96% ethanol		
E1	81.012	3.25
E2	59.033	4.11
E3	59.725	4.05
E4	30.852	6.05
M: Extraction with 80% ethanol		
M1	83.327	4.58
M2	127.683	3.98
M3	43.898	5.18
M4	52.846	4.98
A: Extraction with 70% acetone		
A1	67.200	3.21
A2	56.612	5.89
A3	3.064	7.11
A4	37.602	3.05
Eth: Extraction with [(Eth:EtOH:H_2O),(25:3:1)].		
Eth1	0.112	6.21
Eth2	0.040	7.28
Eth3	0.040	7.98
Eth4	0.016	6.85

No: 1-3 for *Q.infectoria* subp. *infectoria*, No: 4 for *Q.infectoria* subsp. *boissieri

A chromatogram of the standart and sample are shown in Figures 2 and 3, respectively.

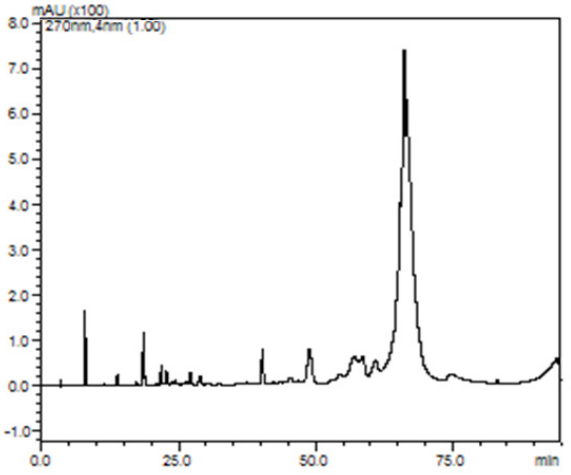

Figure 2. HPLC chromatogram of tannic acid.

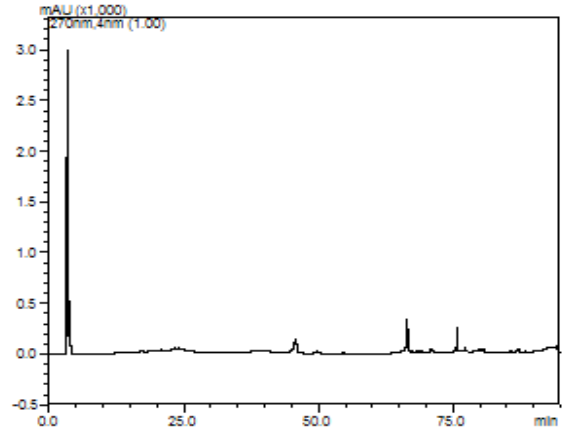

Figure 3. HPLC chromatogram of ethanolic extract of Q.*infectoria* subsp. *infectoria*.

Table 2 depicted the most quantity of tannic acid has been determined as 81.012 mg/g in the small grapegalls of *Q.infectoria* subsp. *infectoria* (sample number E1) for all %96 ethanolic extracts (E1-E4). Medium grapegalls of *Q.infectoria* subsp. *infectoria* (sample number M2) has the most amount of tannic acid in the extraction with 80% ethanol. But the smallest grapegalls of *Q.infectoria* subsp. *infectoria* (sample no:A1) has the most amount of tannic acid in the extraction with 70% acetone; All samples of *Quercus* species contained tannic acid between 0.040 and 0.112 mg/g in the extraction with diethylether: ethanol: water mixture, (25:3:1)). Even, it has been determined that all samples of *Q.infectoria* subsp. *boissieri* have the least amount of tannic acid (0.040-30.852 mg/g) for all extracts except extraction with (diethylether:ethanol:water) mixture. Also, it has been shown that tannic acid contents of grapegalls have more than applegalls in most extracts. So, tannic acid has been determined in the 80% ethanolic extract of *Quercus infectoria* subsp. *infectoria* as the most quantity in all samples. This is the first study on the quantitative determination of tannic acid for these *Quercus* subspecies in literature**.** Verzele *et al***.** (1983) have reported four commercial tannic acid isolated from different plant extracts. These commercial tannic acid include not only tannic acid but also very little quantity of gallic acid. So, the tannic acid and gallic acid percentages (%) of them have been analyzed by HPLC in 1983. In this study; sumac leaves, some galls or leaves from *Rhus species* and Tara pods has been selected for plants. Also, hexane (A) and methanol: tetrahydrofuran (75:25) containing citric acid (0.25 %) were used for mobile phase by a gradient programme on 5µm ROSİL column in Normal Phase HPLC mode. Also, the gallic acid and tannic acid percentages in *Q.infectoria* galls has been determined as (0.6%) and (92.05 %), respectively. 5µm-ROSİL-C18-D column and water with 0.5 phosphoric acid (A) and methanol (B) have been applied for Reversed Phase HPLC (RP-HPLC) mode in the same study. Gallic acid and tannic acid percentages has been detected as (0.2%) and (91.00%) respectively in *Q.infectoria* gall by RP-HPLC analysis. On the other hand, Hamad *et al***.** (2017) have determined the quantities (as mg/g) of nutgalls and roots of *Q.infectoria* for tannic acid in their methanolic extracts as 91.42 mg/g and 0.11 mg/g, respectively.

CONCLUSION

This is the first study on quantitative determination of tannic acid for *Quercus infectoria* subsp. *boissieri* and *Q.infectoria* subsp. *infectoria* in literature. Even, it has been determined that maceration with %80 ethanol is the best extraction method for obtaining the highest quantity of tannic acid in all extraction methods. So, our study will lead the determination of tannic acid contents in other plants in future. Even, screening of new tannic acid sources is important in pharmaceutical area. Finally, we intend to continue this study with the other phenolic compounds and also their bioactivity trails in the future.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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