



MEDICINAL COMPOUNDS OF *QUERCUS* BARK AND RELATED AGRICULTURAL AND PHARMACEUTICAL APPLICATIONS

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Received 28 February, 2019,

Accepted 17 March, 2019

ABSTRACT

Identifying phenols in ornamental trees may provide sources of natural compounds that have applications in the agricultural and pharmaceutical industries. In this study, we profiled phenolic acids in the bark of *Quercus* sp. using HPLC-DAD. *Q. robur* showed high ellagic acid (in *Q. robur*). *Q. macrocarpa* had high caffeic acid. All species showed antibacterial and antifungal activities. *P. aeruginosa* was the most sensitive species for bark extracts. The antifungal activities were high against *A. flavus*. The study revealed new natural sources of phenolic acids that have antimicrobial activities with agricultural and pharmaceutical applications.

Keywords: *Quercus*, antibacterial, antifungal.

INTRODUCTION

The *Quercus* belonging to the *Fagaceae* family are trees widely distributed all over the world and estimated to contain 450 species (Sanchez-Burgos et al 2013). There are differences in their morphological appearance and chemical composition and the best-known species in Europe is *Quercus robur* L. (eng. common oak). This plant is naturally growing in Europe, China and North

America and used in traditional medicine for the treatment of diarrhea and inflammations. The oak bark raw material is described as the cut and dried bark of young branches and lateral shoots which contain a minimal amount of 3% of tannins which is composed of either: galloyl esters and their derivatives (gallotannins, ellagitannins and complex tannins) or oligomeric and polymeric proanthocyanidins and can possess different interflavanyl coupling and substitution patterns (Niemetz and Gross, 2005 and Bobinac et al 2012). However, there is no information in literature about the phenolic profile of bark extracts of this species. Drózdź and Pyrzynska (2018) reported that *Q. robur* bark from Poland have strong antioxidant activities, however, they did not describe the phenolic composition and their respective bioactivities.

Quercus acutissima Carruth. (eng. sawtooth oak) is another naturally growing species, native to eastern and southern regions of Asia and has become naturalized in some eastern regions of North America. The use of *Q. acutissima* as medicinal plant was mentioned in traditional Asian medicine, especially bark extracts that are used for the treatment of skin disorders (Tanaka et al 1995 and Koseki et al 2015).

Quercus macrocarpa Michx. (eng. bur oak) is native to North America and tolerate drought conditions (Tang and Kozlowski, 1982). Little is

known about *Q. macrocarpa* bark chemical composition and possible bioactivities. In general, *Quercus* genus is widespread distributed all over the world, with potentially bioactive raw material used in specific regions as traditional medicine, but the information from experimental studies regarding the bioactivity of the bark extracts is limited.

In the current study, we investigate the phenolic and bioactivities of different species of oak to explore the possible applications in the agricultural and pharmaceutical industries.

MATERIALS AND METHODS

1. Plant material and cell cultures

The bark of *Q. acutissima*, *Q. macrocarpa* and *Q. robur* (*Fagaceae* family) was obtained from the Arboretum of the University of Guelph, Ontario, Canada. The species were identified and vouchered by Hosam Elansary at the University of Guelph. The fresh bark extracts of 0.25 g was dried in the oven at 35°C until reaching constant weight. Dried bark was ground then dissolved in methanol (3 mL, 99%) for 1 h at room temperature in the dark. The samples were centrifuged at 10,000 rpm (7000 × *g*) for 5 min to separate the supernatant (~2.7 mL) from the precipitate. The samples were stored at -80°C for future analyses. An analytical/HPLC grade chemicals were used (Sigma Aldrich, Germany) for the bioassay. The bacterial and fungal cultures were obtained from the Department of Floriculture and Ornamental Horticulture, Faculty of Agriculture, Alexandria, Egypt.

2. Analyses of phenolic compounds

Quercus bark samples were dried by lyophilization (Labconco, USA) then powdered. The plant samples (0.5 g) were extracted following the procedure described by Szopa et al (2017). The methanolic extracts were subjected to chromatographic analyses by a modified validated HPLC method (Ilnain-Wojtaszek and Zgorzka, 1999; Sułkowska-Ziaja et al 2017). An HPLC-DAD (Merck-Hitachi) apparatus and a Purospher® RP-18e analytical column (4 × 250 mm, 5 mL; Merck) were used for the analyses. The flow rate of 1 mL/min (gradient program), injection volume of 10 µL and detection wavelength (254 nm) were used (Szopa et al 2016, 2017, 2012). The quantification was carried out by comparing the UV-DAD spectra and *t_r* values with that of commercially available

standards of phenolic acids. The following standards were used for phenolic acids quantification: benzoic acid and its derivatives: 3,4-dihydroxyphenylacetic acid, ellagic acid, gallic acid, gentisic acid, *p*-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, vanillic acid; cinnamic acid and its derivatives: caffeic acid, *o*-coumaric acid, *m*-coumaric acid, *p*-coumaric acid, ferulic acid, hydrocaffeic acid, isoferulic acid, sinapic acid, and depsides: chlorogenic acid, neochlorogenic acid and rosmarinic acid.

3. Antibacterial activity

Bark extracts antibacterial activities were screened against *Bacillus cereus* (ATCC 14579), *Escherichia coli* (ATCC 35210), *Listeria monocytogenes* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 6538) using the microdilution method (Elansary et al., 2018). Microtiter plates (96-well, Pharma, Japan) contained known concentration of bark extract in each well mixed with bacterial inoculum (1.0 × 10⁴ CFU per well) in 100 µL tryptic soy broth were incubated at 37°C for 24 h in a rotary shaker (Pharma, Japan). The minimum inhibitory concentration (MIC) was defined as the lowest concentration of plant extract that exhibited no visible growth using binocular microscope and was determined following the incubation period of the microtiter plates. The minimum bactericide concentration (MBC) defined as the lowest concentration that caused no visible growth which indicated killing of 99.5% of the inoculum was determined using serial subculturing of bark extracts (2 µL). A wave length of 655 nm was used to determine the optical density (OD) in a spectrophotometer. A positive control was used (streptomycin, 0.01-10 mg/mL) as well as the negative one (DMSO, 5%).

2.4. Antifungal activity

The antifungal activities of bark extracts were determined against variety of infectious and economic fungi including *Aspergillus flavus* (ATCC 9643), *A. ochraceus* (ATCC 12066), *A. niger* (ATCC 6275), *Candida albicans* (ATCC 12066), *Penicillium ochrochloron* (ATCC 48663) and *P. funiculosum* (ATCC 56755). The microdilution method was employed in this assay (Elansary et al., 2018) using bark extract (2 µL) mixed with broth Malt medium and fungal inoculum (spore suspension concentration of 1.0 × 10⁵) in microtiter

plates. The plates were incubated at 28°C for 72 h in a rotary shaker then the minimum inhibitory concentration (MIC) was determined as the lowest concentration inhibiting fungal growth at the binocular microscopic level. The minimum fungicidal concentration (MFC) was defined as the minimum concentration showing no visible growth and indicating the killing of 99.5% of the original inoculum. MFC was determined using a serial sub-cultivations of the bark extracts (2 µL) added to 100 µL of broth and inoculum, then incubated at 28°C for 72 h. A positive control was used (ketoconazole, 1–3500 µg/mL).

5. Statistical analyses

The Least Significance Difference (LSD) was determined in the SPSS software (version 22.0). The quantitative results of chromatographic analyses were expressed in [mg 100g⁻¹] D.W. (dry weight) as the mean ± standard deviation (SD) of three series of experiments.

RESULTS

1. HPLC-DAD

3.1. In *Q. acutissima* bark, there were caffeic acid, ellagic acid, gallic acid and protocatechuic acid (**Table 1**). The dominant compound was the ellagic acid and was followed by the gallic acid. The amounts of protocatechuic and caffeic acids were lower. In *Q. macrocarpa*, high amount of caffeic acid was found as well as other phenolic acids including ellagic, protocatechuic and gallic acid. In *Q. robur*, four phenolic acids were found including: ellagic acid, gallic acid, protocatechuic acid and vanillic acid. Ellagic acid (97.82 mg 100g⁻¹ DW) while the amounts of other compounds were lower: gallic acid – 8.23 mg 100g⁻¹ DW, protocatechuic acid – 6.96 mg 100g⁻¹ DW and vanillic acid – 2.61 mg 100g⁻¹ DW.

Table 1. The phenolic acid compositions of *Q. acutissima*, *Q. macrocarpa* and *Q. robur* outer bark extracts

<i>Quercus</i> species	Chemical compound	Amount [mg 100g ⁻¹] D.W.
<i>Q. acutissima</i>	Caffeic acid	4.30 ± 0.05
	Ellagic acid	13.50 ± 2.84
	Gallic acid	7.09 ± 0.59
	Protocatechuic acid	5.39 ± 0.76
<i>Q. macrocarpa</i>	Caffeic acid	100.58 ± 18.02
	Ellagic acid	5.07 ± 0.05
	Gallic acid	0.87 ± 0.03
	Protocatechuic acid	3.36 ± 0.02
<i>Q. robur</i>	Ellagic acid	97.82 ± 1.74
	Gallic acid	8.23 ± 0.39
	Protocatechuic acid	6.96 ± 1.14
	Vanillic acid	2.61 ± 0.15

2. Antibacterial activities

The antibacterial activities of the bark extracts of *Q. acutissima*, *Q. macrocarpa* and *Q. robur* using the microdilution methods are shown in **Table 2**. The three extracts showed clear antibacterial activities against most species studies. The MIC values ranged between 0.05 and 0.29 mg mL⁻¹ while the MBC ranged between 0.11 and 0.66 mg mL⁻¹. The response of the bacterial species to the extract used varied among species. The highest antibacterial activities were found in the extracts of *Q. robur* compared to the other two species. The three extracts showed higher antibacterial activities against *P. aeruginosa*, *M. flavus* and *E. coli* compared to other bacterial species. Further, their antibacterial activities were comparable to antibiotics.

3. Antifungal activities

The extracts were screened for their antifungal activities against several fungi as shown in **Table 3**. The MIC ranged between 0.16 and 2 mg mL⁻¹ while the MFC ranged between 0.23 and 3.61 mg mL⁻¹. There were obvious antifungal activities against some fungi such as *A. flavus*, *P. funiculosum* and *P. ochrochloron*. However, *A. ochraceus* and *A. niger* as well as *C. albicans* showed slight resistance to the extracts. The activities of the extracts were comparable to commercial reagents in most cases.

Table 2. Minimum inhibitory (MIC) and bactericidal concentration (MBC) of *Q. acutissima*, *Q. macrocarpa* and *Q. robur* outer bark extracts (mg mL⁻¹) as well as phenolic standards.

	<i>Pseudomonas aeruginosa</i> MIC MBC	<i>Bacillus cereus</i> MIC MBC	<i>Listeria monocytogenes</i> MIC MBC	<i>Escherichia coli</i> MIC MBC	<i>Micrococcus flavus</i> MIC MBC	<i>Staphylococcus aureus</i> MIC MBC
<i>Q. acutissima</i>	0.09 ± 0.01 0.18 ± 0.02	0.17 ± 0.01 0.37 ± 0.03	0.27 ± 0.02 0.66 ± 0.03	0.17 ± 0.01 0.32 ± 0.02	0.17 ± 0.01 0.41 ± 0.03	0.23 ± 0.01 0.46 ± 0.01
<i>Q. macrocarpa</i>	0.07 ± 0.01 0.15 ± 0.01	0.16 ± 0.01 0.35 ± 0.03	0.29 ± 0.01 0.62 ± 0.02	0.13 ± 0.01 0.29 ± 0.02	0.14 ± 0.01 0.34 ± 0.03	0.22 ± 0.01 0.44 ± 0.02
<i>Q. robur</i>	0.05 ± 0.01 0.11 ± 0.01	0.11 ± 0.01 0.27 ± 0.02	0.25 ± 0.01 0.53 ± 0.03	0.10 ± 0.01 0.21 ± 0.02	0.10 ± 0.01 0.20 ± 0.02	0.23 ± 0.02 0.45 ± 0.01
ellagic acid	0.04 ± 0.01 0.10 ± 0.01	0.09 ± 0.01 0.22 ± 0.01	0.23 ± 0.01 0.49 ± 0.02	0.09 ± 0.01 0.19 ± 0.03	0.09 ± 0.01 0.18 ± 0.01	0.20 ± 0.01 0.41 ± 0.03
caffeic acid	0.06 ± 0.01 0.13 ± 0.01	0.13 ± 0.01 0.29 ± 0.01	0.27 ± 0.01 0.58 ± 0.03	0.11 ± 0.01 0.25 ± 0.01	0.13 ± 0.01 0.30 ± 0.02	0.20 ± 0.01 0.41 ± 0.03
Streptomycin	0.08 ± 0.01 0.16 ± 0.01	0.07 ± 0.03 0.15 ± 0.01	0.14 ± 0.01 0.29 ± 0.03	0.12 ± 0.01 0.27 ± 0.01	0.11 ± 0.01 0.21 ± 0.02	0.19 ± 0.01 0.32 ± 0.01

Table 3. Minimum inhibitory (MIC) and fungicidal concentration (MFC) of *Q. acutissima*, *Q. macrocarpa* and *Q. robur* outer bark extracts (mg mL⁻¹) as well as phenolic standards.

	<i>Aspergillus flavus</i> MIC MFC	<i>Aspergillus ochraceus</i> MIC MFC	<i>Aspergillus niger</i> MIC MFC	<i>Candida albicans</i> MIC MFC	<i>Penicillium funiculosum</i> MIC MFC	<i>Penicillium ochrochloron</i> MIC MFC
<i>Q. acutissima</i>	0.24 ± 0.01 0.51 ± 0.03	0.26 ± 0.02 0.57 ± 0.02	0.21 ± 0.01 0.41 ± 0.02	0.40 ± 0.02 0.86 ± 0.03	0.38 ± 0.02 0.69 ± 0.03	0.25 ± 0.01 0.52 ± 0.02
<i>Q. macrocarpa</i>	0.22 ± 0.02 0.43 ± 0.01	0.24 ± 0.03 0.48 ± 0.02	0.21 ± 0.01 0.40 ± 0.03	0.34 ± 0.03 0.76 ± 0.03	0.29 ± 0.03 0.68 ± 0.03	0.21 ± 0.02 0.43 ± 0.03
<i>Q. robur</i>	0.19 ± 0.02 0.40 ± 0.02	0.26 ± 0.01 0.53 ± 0.03	0.16 ± 0.01 0.35 ± 0.02	0.31 ± 0.01 0.62 ± 0.03	0.26 ± 0.01 0.63 ± 0.03	0.16 ± 0.01 0.33 ± 0.03
ellagic acid	0.15 ± 0.01 0.33 ± 0.03	0.22 ± 0.03 0.45 ± 0.03	0.13 ± 0.01 0.28 ± 0.01	0.30 ± 0.03 0.61 ± 0.03	0.23 ± 0.02 0.51 ± 0.03	0.12 ± 0.01 0.25 ± 0.01
caffeic acid	0.20 ± 0.01 0.40 ± 0.01	0.22 ± 0.01 0.45 ± 0.01	0.20 ± 0.01 0.38 ± 0.01	0.32 ± 0.01 0.64 ± 0.03	0.27 ± 0.01 0.62 ± 0.02	0.20 ± 0.03 0.42 ± 0.01
KTZ	0.21 ± 0.01 0.41 ± 0.01	0.21 ± 0.01 0.42 ± 0.02	0.12 ± 0.01 0.23 ± 0.01	0.20 ± 0.01 0.42 ± 0.01	2.00 ± 0.10 3.61 ± 0.03	0.21 ± 0.01 0.42 ± 0.01

DISCUSSION

The HPLC-DAD analyses of methanolic extracts of the bark of three *Quercus* species indicated that specific phenolic acids are the major active ingredients. Three phenolic acids were common in all three bark extracts (ellagic acid, gallic acid and protocatechuic acid) and they are benzoic acid derivatives. Ellagic acid and gallic acid are known derivatives produced in tannin hydrolyses, typical for *Quercus* species (Niemetz and Gross, 2005).

Interestingly, extreme high amount of ellagic acid were found in *Q. robur* bark extract (97.82 mg

100g⁻¹ DW), that was 7-times more than in *Q. acutissima* and 17-times more than in *Q. macrocarpa* bark extracts (Table 1). In *Q. acutissima* and *Q. macrocarpa* bark extracts, there was noticeable amount of caffeic acid. In *Q. macrocarpa*, the caffeic acid amount was very high – 100.58 mg 100g⁻¹ DW (23-times more than in *Q. acutissima*) (Table 1). In *Q. robur* bark extract, the vanillic acid was detected (Table 1). This phenolic acid wasn't detected in other *Quercus* species. In the *Quercus* species, the most often studied bioactive metabolite content was in the leaves and needles extracts of *Q. robur*. (Kuiters et al 1986). They found some

phenolic acids including: *p*-hydroxybenzoic acid, vanillic acid, gallic acid, syringic acid, ferulic acid and *o*- and *p*-coumaric acids. For *Q. acutissima*, the gentisic acid (phenolic acid) was confirmed in the extracts of fresh acorns (Ishimaru et al 1987). However, in the available literature we couldn't find information about phenolic acid estimation in *Q. macrocarpa*, and in the bark extracts of the three *Quercus* species. That is important, because that cortex is recognized as oak's raw material. Our study showed differences in secondary metabolites composition between examined cortex extracts and for the first time the phenolic acids profile is revealed in these materials.

The detected phenolic acids in the studied extracts are very important from pharmacological and economic point of view. For example, gallic acid has antibacterial, hypoglycemic, anti-inflammatory and antimutagenic activities (Khadem and Marles, 2010). Ellagic acid is known for strong antioxidative, antiproliferative and anti-cancer properties (Seeram et al 2005). Protocatechuic acid show antifungal, antibacterial, antiviral, anti-inflammatory, antiatherosclerotic, antiulcer and anticancer properties (Khadem and Marles, 2010; Kedzierska et al 2012 and Kakkar & Bais, 2014). Vanillic acid also shows antioxidant and additionally hepatoprotective actions (Brand-Williams et al 1995 and Itoh et al 2010). The presence of these compounds, beside tannins, is contributing to the pharmacological actions of these raw materials.

The *Quercus* cortex is recognized in phototherapy as valuable plant raw material due to extreme high content of tannins (Kraus et al 2003; European Directorate for the Quality of Medicines, 2017). There is little is known about these secondary metabolites in the bark extracts of *Q. acutissima* and *Q. macrocarpa*. Only the presence of catechin in was described before in *Q. acutissima* (Tanaka et al 1995 Ishimaru et al 1987).

Drózdź and Pyrzyńska (2018) reported strong antioxidant activities in *Q. robur* bark from Poland, however, they did detect the phenolic profile of these trees. A recent investigation on *Q. robur* and *Q. petraea* leaves, twigs, and acorns from Serbia revealed strong antioxidant activities (Sanchez-Burgos, 2013). *Q. macrocarpa* showed higher antioxidant activities than *Q. acutissima* and this could be explained by the extreme high ratio of the caffeic acid in *Q. macrocarpa*. Caffeic acid is known for antioxidant, antibacterial and antifungal activities (Chong et al 2009).

The three extracts showed antibacterial activities against most species studies and the highest

antibacterial activities were found in the extracts of *Q. robur* compared to the other two species. Previous report on *Q. robur* from Finland found some antibacterial activity of the bark extract on *S. aureus* and *C. albicans* using the agar diffusion method (Andrenšek et al 2004). In the current study, we found strong antibacterial activities against *P. aeruginosa*, *M. flavus* and *E. coli* and moderate activities against other bacterial species. The work on *Q. robur* bark bioactivities is relatively limited but other species revealed some antibacterial activities against *Chromobacterium violaceum* such as *Q. cortex* (Deryabin and Tolmacheva, 2015). This strong antibacterial activities might be attributed to the Ellagic acid which has some antibacterial activities against certain bacteria such as *Streptococcus mutans*, *Streptococcus sanguis* and *Streptococcus salivarius* (Loo et al 2010). Further, green tea catechins has been associated with a strong antifungal activities against *C. albicans* (Steinmann et al 2013). In the current study, we found obvious antifungal activities against *A. flavus*, *P. funiculosum* and *P. ochrochloron* as well as moderate activities against *C. albicans*. Such activities is mainly attributed to ellagic acid and caffeic acid which are the main components found in these plants bark.

CONCLUSIONS

The study revealed the availability of several phenolic acids in high amounts such as the ellagic acid (in *Q. robur*), caffeic acid (in *Q. macrocarpa*) and catechins in the three species. The three oak bark extracts showed clear antibacterial activities against most bacteria used. The highest antibacterial activities were found in the extracts of *Q. robur* and the three extracts showed higher antibacterial activities against *P. aeruginosa*, *M. flavus* and *E. coli* compared to other bacteria. There were obvious antifungal activities against some fungi such as *A. flavus*, *P. funiculosum* and *P. ochrochloron*. Oak barks used in this study are valuable sources for antimicrobial compounds.

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المركبات الطبية في قلف البلوط وعلاقتها بالتطبيقات الزراعية والطبية

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Received 28 February, 2019,

Accepted 17 March, 2019

الموجز

أظهرت نشاطا مضادا للبكتريا والفطريات. وجد ان البكتريا من النوع *P. aeruginosa* كانت الأكثر حساسية لكل مستخلصات أنواع القلف. النشاط المضاد للفطريات كان مرتفعا ضد فطريات *A. flavus*. أثبتت الدراسة توافر مصادر طبيعية جديدة للاحماض الفينولية والتي لها نشاط مضاد للميكروبات ولها تطبيقات زراعية وصيدلانية.

الكلمات الدالة: البلوط، النشاط المضاد للبكتريا، النشاط المضاد للفطريات

التعرف على الفينولات في نباتات الزينة يمكن ان يمدنا بمصدر للمركبات الطبيعية التي لها تطبيقات في الصناعات الزراعية والصيدلانية. تم في هذه الدراسة تحديد الاحماض الفينولية في قلف أشجار البلوط باستخدام تقنية HPLC-DAD. ووجد ان أشجار البلوط من النوع روبر بها محتوى مرتفع من حمض الإلاجيك، كما ان أشجار البلوط من النوع ماكروكاربا بها محتوى مرتفع من حامض الكافيك. الأنواع الثلاثة

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