

Oak (*Quercus aegilops* L.) Root Bark Tannins Astringency, Antioxidant Potential, and Use as Functional Tea

Ayed S. Amr¹, Mousa N. Ahmad¹, Mai A. Abdullah²

¹ Professor of food science and human nutrition respectively/ Dept. of Nutrition and Food Technology/ University of Jordan/ Amman 11942

² Assistant professor of food science/ Al-Balqa Applied University/Al-Hosn/ Jordan.

Received on 23/12/2019 and Accepted for Publication on 12/10/2020.

ABSTRACT

Tannins from the root bark of *Quercus aegilops* L. were extracted in two successive seasons and their astringency score and antioxidant potential were evaluated using bovine serum albumin (BSA) radial diffusion and 2,2-Diphenyl – 1-Picrylhydrazyl (DPPH) assay methods respectively. The results revealed that the root bark of this oak species contained on average 11.39 % (w/w) tannins as gallic acid, of which about 79% are hydrolyzable (HT) and 21% condensed (CT) tannins with the former composed mainly of ellagitannins and much fewer gallotannins and free gallic acid. The results also revealed that the crude root bark powder and its purified tannins have astringency scores of 15.8 and 138.98 mg BSA/g respectively. The antioxidant potential was evaluated by DPPH assay and expressed as inhibition capacity (IC50) was 149.7 ppm and 19.24 ppm for the crude bark powder and its purified tannins respectively indicating the superior antioxidant potential of these tannins to that of ascorbic acid. The highly-antioxidant functional tea prepared from these tannins was clear and acceptable to tasters.

Keywords: condensed tannins, hydrolyzable tannins, functional foods.

INTRODUCTION

Many plant species are rich in phenolic compounds (Karioti et al., 2010) including tannins (Mammela et al., 2000; Shad et al., 2012) which are a complex and heterogeneous group of water-soluble polyphenolic secondary metabolites (Gangwal, 2013) found in most parts of higher plants and usually have molecular weights varying between 500 and 20,000 Da. (Mane et al., 2007).

Their composition and concentration depend on the species, part, and age of the plant from which they are extracted. Tannins differ from other polyphenols in their ability to complex with and precipitate proteins, alkaloids, carbohydrates, vitamins, and minerals (Marinho et al, 2018, Falcão & Araújo, 2011).

Based on their specific structures and chemical properties, naturally, present tannins are classified by most researchers into two main groups: Hydrolysable Tannins (HT) and Condensed Tannins (CT) (known as

* Corresponding author E-mail: ayedamr@ju.edu.jo.

proanthocyanidins). The former are susceptible to hydrolysis with dilute acid, base, or hot water and consist of ellagi and gallotannins while the latter are not susceptible to hydrolysis and consist of oligomeric or polymeric flavonoid units (catechin or epicatechin) linked via carbon-carbon bonds (Silanikove et al., 2001; Romani et al., 2006; Liu & White, 2012; Lorrain et al., 2013). However, some workers (Gangwal, 2013) suggest a third group i.e., complex tannins which consist of flavan-3-ol units (catechin moiety) glycosidically bound to Gallo or ellagitannin units. Both HT and CT are extracted from plant sources including the bark of wood trees such as oak, chestnut, sumac, and mimosa (Gonultas & Ucar, 2012; Romani et al., 2006) as well as grape seeds (Liu & White, 2012).

Tannins are used in food industries for enhancing the taste of wine, by developing the desirable astringent sensation (Cobzac et al., 2005; Harbertson et al., 2012), as efficient antioxidants for foodstuffs (Shad et al., 2012) and as food preservatives for their antimicrobial activity (Lipińska et al., 2014).

Tannins are also valued for their beneficial role in human health particularly as antidiuretic and anticarcinogenic agents, where they are used in several forms such as dry or as a tea extract (Sakagami et al., 2000; Bawadi et al., 2005; De Wet, 2010; Gonzalez-Castejon & Rodriguez-Casado, 2011; Kumari & Jain, 2012).

On the other hand, naturally occurring tannins in seeds, legumes, and cereals have recently received much attention since they are believed to precipitate and lower the nutritional value and bio-availability of dietary proteins, digestive enzymes, polysaccharides, and minerals especially iron if present in sufficient amounts (Chavan et al., 2001; Ashok & Upadhyaya, 2012). Tannins are also

known for their negative effect on the palatability of plant foods due to complexing with salivary glycoproteins (Padayachee et al., 2017) and the resulting astringent sensation.

The objective of this study is to extract, isolate, identify and characterize the tannins from the root bark of oak trees (*Quercus aegilops* L.) and determine their antioxidant potential, astringency and use in the preparation of a functional tea.

Materials and Methods

Sample collection and preparation: Root samples from 20-year-old Oak trees (*Quercus aegilops* L.) were collected in November/ 2015 from a forest near Amman/ Jordan, washed with distilled water, and air-dried for two weeks at ambient temperature. The bark was stripped of the heartwood and ground using a hammer mill supplied with a 200µm sieve, dried at room temperature to a moisture level of 10%, and stored in air-tight low-density polyethylene bags in the refrigerator as oak root bark powder for further analysis. Triplicate samples were used for all analyses.

Preparation of the crude tannin extract. A 0.5 g portion of oak root bark powder was extracted at room temperature three successive times, each with 10 ml of 80% methanol solution (80:20 v/v, methanol: water) with agitation (Boumerfeg et al., 2018). The extracts were collected and filtered through Whatman no.1 filter paper and the solvent was evaporated at room temperature. The residue was then acidified with two drops of 6 N HCl and taken three times with diethyl ether to remove any fatty material. The diethyl ether was removed using a separatory funnel and the remaining water was removed by the addition of anhydrous sodium sulfate. The residual part containing the crude tannin extract was dissolved in methanol. Triplicate extracts were prepared.

Spot tests for tannin confirmation: Ferric chloride method (Falcão & Araújo, 2011; Sabri et al., 2012; Roshni & Ramesh, 2013) was used to confirm the presence of gallotannins and tannins in general, whereby a bluish-black color indicates the presence of gallotannic acid, green to blue-green indicates the presence of CT and a blue-black color indicates the presence of HT in the form of gallotannins. The nitric acid (pyridine 270970 Sigma-Aldrich) and rhodanine (83700 Fluka) tests were used to confirm the presence of ellagitannins and gallic acid. A progressive formation of blue and pink colors indicates the presence of ellagitannins and gallic acid respectively (Falcão & Araújo, 2011). Condensed tannins were confirmed using the vanillin and acid butanol tests with which they give red and red-orange to red-crimson colors respectively (Falcão & Araújo, 2011). All analyses were carried out in triplicates

Determination of total tannins in oak root powder:

Total tannin content of oak root powder was determined following the Folin-Ciocalteu method as described by Marinho et al. (2018) and Makkar (2000) using Gallic acid (G7384 Sigma-Aldrich) as a standard. Absorbance was measured at 725 nm using a spectrophotometer (Perkin-Elmer Lambda 3B UV/ Vis) and results were expressed as mg gallic acid equivalent (eq.)/Kg (ppm) of oak root powder and reported as mean values with \pm SEM. All analyses were carried out in triplicates.

Total hydrolysable tannins in the methanolic tannin extract:

The total HT expressed as tannic acid was spectroscopically determined on the tannin -methanolic extract as described by Hartzfeld et al. (2002). The absorbance was measured at 550 nm after 2 minutes of tempering the solution at 30°C using Perkin-Elmer Lambda 3B UV/ Vis spectrophotometer (Saad et al., 2012) using tannic acid (Sigma- Aldrich 16201) as a standard. Results were expressed % w/w of dry oak bark powder. All analyses were carried out in triplicates and the results were reported as means \pm SEM.

Determination of total gallotannins and total and free gallic acid:

Free gallic acid in oak bark powder was determined on the non-hydrolyzed bark sample by the Rhodanine (Sigma- Aldrich, R 4375) method as described by Khoddami et al. (2013) and Hartzfeld et al. (2002) and expressed as ppm of root powder. Total gallic acid was determined after hydrolysis of the root bark sample with H₂SO₄ following the same procedure. In both cases gallic acid (Sigma- Aldrich G7384) was used as a standard and absorbance was taken at 520 nm using Perkin-Elmer Lambda 3B UV/ Vis spectrophotometer. Gallotannins as % w/w were calculated by subtracting the free gallic acid from the total gallic acid. Analyses were carried out in triplicates and the results were reported as means \pm SEM.

Ellagic acid:

Ellagic acid was determined according to the method described by Engström, et al. (2015) which is based on the formation of quinine oxime measured spectrophotometrically at 538 nm using ellagic acid (E2250 Sigma-Aldrich) as a standard. Results were expressed as % w/w of powder and all analyses were carried out in triplicates and the results were reported as means \pm SEM.

Total condensed tannins (proanthocyanidins):

For comparison purposes, two conventional methods were used to estimate the condensed tannins in oak root bark powder; In the first, condensed tannins were determined by the butanol- HCl method as described by Boumerfeg et al. (2018) where the absorbance was read at 530 nm using Perkin-Elmer Lambda 3B UV/Vis spectrophotometer, and results were expressed as % w/w cyanidin eq. of root bark powder. The other method used is the vanillin assay, which is based on the principle of HCl catalyzed condensation of vanillin with the phloroglucinol ring (Boumerfeg et al., 2018) and the formation of a colored compound with absorbance at 500nm (Hummer & Schreier, 2008). Results were expressed as % w/w catechin eq. of oak bark powder

using catechin (C1251 Sigma-Aldrich) as standard. All analyses were carried out in triplicates and the results were reported as means \pm SEM.

Tannin astringency/ radial diffusion assay: This test was performed as described by Guimarães-Beelen et al. (2006) and Hagerman (2002). The tannins that reacted with the Bovine Serum Albumin (BSA) were estimated by the size of the ring formed after incubation for 96 hours at 35°C. The tannic acid standard was used as a positive control for comparison. Astringency mg (BSA)/ g of oak root bark powder = (volume (ml) x protein concentration (mg/ ml) / applied sample weight). The volume was determined by multiplying the height of the agar with the length of the radius before and after incubation. Analyses were performed in triplicates, and the results were reported as Mean \pm SEM.

Radical scavenging capacity (the antioxidant activity):

The antioxidant activity of the methanolic extracts of bark powder and tannins was evaluated by using the DPPH (2,2-diphenyl-1-picrylhydrazyl (281689 Aldrich)) method as described by Gülcin (2012). Sanchez-Moreno (2002) and Zhou *et al.* (2011). DPPH% inhibition = $[(A1 - A2)/A1] \times 100$ where A1 = the absorbance of the control (methanol) and A2 = the absorbance of the sample. The IC50% value is the concentration (ppm) of the crude powder of purified tannins which results in 50% inhibition of DPPH (Shukla et al., 2009) as calculated from the regression equation describing the % DPPH inhibition vs. concentration of the crude oak root powder or purified tannins in methanol. The analysis was carried out in triplicates.

Preparation of functional tea and sensory evaluation:

For this part of the work, oak root bark tannins were extracted from oak root bark three successive times, each with 10 times their weight of water, filtered through Whatman #40 filter paper then freeze-dried using Operon FDB5502 freeze drier (Korea). The fluffy powder was then weighed and mixed with black tea at a level of 30% (w/w)

and packed in tea bags. The so-prepared functional tea was tested for its acceptance using attribute diagnostic test (Munoz, 2013) which included nine ordered categories ranging from (9) Like extremely to (1) Dislike extremely. The evaluation was performed in 2 sessions (2 replicates) with the same 25 semi-trained, randomly chosen panelists in each session. Panelists were from both genders and tasting took place in a specialized sensory analysis laboratory. Each subject was presented with a tray with a cup of boiled water, a bag of functional tea, sugar, and a teaspoon and asked to prepare their tea and evaluate it for color, odor, taste, transparency, and overall acceptability. Sugar was presented to mask any objectionable taste or bitterness as the tea would be used for medicinal purposes. Analysis of variance was performed on the data using ANOVA/ GLM procedure using a SAS program (SASI, 2000) to elucidate the significance of the effects of replicates and panelists on each of the tested sensory parameters, and scores were tallied and calculated as % of responses \pm SEM.

Results and discussion

Qualitative colorimetric analysis for tannins in oak

root bark: Table 1 shows the results of the spot colorimetric tests on the crude methanolic tannin extracts from the oak root bark. FeCl₃ reagent gave a dark bluish to black color, indicating the presence of phenolic compounds in general rather than for tannins specifically (Falcao & Araujo, 2011). Positive vanillin reaction was also observed indicating that the root bark extract contained CT, the intensity and dispersion of red color was low compared to the progression of dark crimson and red colors formed when vanillin reagents were added to the standards of catechin and epicatechin respectively. The Acid-butanol test is used as a qualitative test for CT (Falcao & Araujo, 2011), it is based on the hydrolysis of proanthocyanidins using n-butanol/ HCl reagent (Amarowicz and Pegg, 2006). The formation of red color indicates the presence of CT in the oak root bark extract. The nitrous acid test is specific for ellagitannins in the form of released ellagic acid based on the formation of the quinine oxime of the ellagic

acid nitrosylation product (Engström, 2015). Screening for ellagic acid in oak bark extractives was positive, with a blue to purple color formation indicating the presence of ellagic acid.

Rhodanine test is based on the hydrolysis of gallotannins into gallic acid monomers, rhodanine reacts with the vicinal hydroxyl groups of free gallic acid producing a red complex (Khoddami et al., 2013; Phakthong et al., 2014). Rhodanine test resulted in pink color formation indicating a positive reaction and thus the presence of gallic acid in a free form.

This is the first report on the qualitative phytoscreening analysis of the tannins from the root bark of *Quercus aegilops* L. or oak root at large, however, these results conform with those reported by other workers on plant phenols (Boumerfeg et al., 2018) as well as studies on the bark and heartwood of other oak species (Zhang et al., 2015). The same results were obtained from the three replicates.

Total tannins: total tannins by the Folin-Ciocalteu method was on the average 11.39 % (w/w) as gallic acid compared to 4.0-16.0 % (w/w) reported by Sánchez-Rangel *et al.* (2013) as catechin and galocatechin in the stem of Oak (*Quercus pedunculata* Ehrh.) bark. Our results are close to theirs although our results are expressed as gallic acid. Tannin levels in trees are usually at their maximum during the spring and summer (Ncube et al., 2014) when the activity of insects is highest and the tannins play the role of defense against such predators. Tannin content also varies according to plant part and age as well as time after felling (Falcão & Araújo, 2011; Feng et al., 2013; Shad et al., 2012). The method of analysis is another source of variation in the results, as the biochemical basis of each method determines not only the form on which the results are expressed but also their level.

Total condensed tannins: CT levels in oak root bark were on average 2.05% (w/w) as catechin and 1.64 % (w/w) as cyanidin by the vanillin and butanol- HCl methods respectively, which is an average of about 21% of the total

tannins. Catechin has a molar mass of 290.26 which is almost equal to the 287.24 of cyanidin. The difference between the two values can be attributed to the methods of analysis. Our results are consistent with the findings of other researchers (Hummer & Schreier, 2008) to the effect that the results of the two methods are not always identical and that each method has its drawbacks. Currently, the vanillin method has been replaced with the acid-butanol method because catechin, used as a standard for the vanillin method, results in overestimation of the proanthocyanidin (Hummer & Schreier, 2008). Moreover, the vanillin method measures both monomeric (non-tannin compounds) in addition to the polymeric flavonoids causing the lack of reproducibility (Sharma et al., 2015) as well as the higher observed value than that of the butanol-HCl method.

Total hydrolysable tannins: HT is sub-classified into gallo and ellagitannins (Sakagami et al., 2000). Our results show that the level of this group in our sample is 9.02 % (w/w) i.e., about 79% of tannins as tannic acid which makes them the major form of tannins (Table 2) in *Quercus aegilops* L.

The results also show that they consist of 0.82 % (w/w) free gallic acid, 1.88 % (w/w) gallotannins, and 6.01 % (w/w) ellagitannins as ellagic acid which makes the latter major constituent (i.e., 66.6% of total HT) of the HT in this oak species. The sum of the three forms is close to the total HT (Table 2) notwithstanding the difference in the forms in which they are presented due to the different assay methods used for each form. This finding conforms with those of other workers to the effect that hydrolysable tannins represent the majority of the tannins of North American white oak (*Quercus alba*) and European red oak (*Quercus robur*) (Mammela et al., 2000) and mango (*Mangifera indica* L.) peels (Glabasnia et al., 2006; Sáyago-Ayerdi et al., 2013); and that ellagitannins are their major constituent.

Tannin astringency/ radial diffusion assay: Astringency of a substance is evaluated by its capacity to precipitate protein (Guimarães-Beelen et al., 2006). It was measured in this study by the radial diffusion method based

on diffusion of tannins through an agarose gel to form a ring of precipitate which allows the estimation of functional activity rather than the chemical identification of tannins (Zhang et al.,2015).

Proanthocyanidins (CT) can precipitate proteins at pH values near their isoelectric point (4.6) due to strong hydrogen bonding with them (Marinho et al.,2018). Proteins with high proline content have higher affinities for tannins compared to those with lower proline levels, due to their open conformation and ability to form strong hydrogen bonds especially with CT (Quideau,2011), therefore the bovine serum albumin (BSA) is used for precipitation assays due to its high proline content (Quideau,2011, Hummer & Schreier,2008). In this study, the astringency score of the tannic acid standard was 133 ± 0.03 mg BSA/ g tannic acid, while that of the bark powder was 15.83 ± 0.01 mg BSA /g powder or about 11.90% of tannic acid activity. However, since the total tannin content of the oak root powder is about 11.39 % (w/w) (Table 2) it means that the calculated astringency score of the root bark tannins is 138.9 which is higher than that of the standard tannic acid.

DPPH radical scavenging capacity (antioxidant potential): Our results reveal that by applying the regression equation for % inhibition vs concentration (Fig. 1), the IC₅₀ for crude oak root bark powder is 149.7 ppm and that of the oak root tannins themselves was 19.2 ppm (Fig. 1). However, since our root bark sample contains only 11.39%(w/w) tannins, then taking into consideration the dilution factor, the calculated theoretical value IC₅₀ of the oak root tannins from the oak root bark powder is 16.9 ppm which is close to that of the actual value of the root bark tannins (i.e. 19.2) and lower than IC₅₀ of 26.68 of ascorbic acid (Shukla *et al.*, 2009). These values not only indicate the high antioxidant power of these tannins but also confirm the accuracy of the method used in extraction and analysis. Since the lower the IC₅₀ of the antioxidant the higher its antioxidant potential by DPPH inhibition, it is clear that the antioxidant potential of the oak root tannins is superior to that of ascorbic acid and that it may be used as

a natural and available source of antioxidants. These findings agree with those reported by Andrenseka et al. (2004) on the species of *Quercus robur* to the effect that the oak cortex materials have high potential antioxidative activity. Zhang et al.,2015 reported that oak tannins exhibited antioxidant properties through different mechanisms of action including the ability to reduce the oxygen availability, chelate metals, scavenge free radicals, and inhibit the free radical formation through possible combination with quinones. The same authors reported that ellagitannins possess powerful inhibition capability for peroxidation at low concentrations (less than 5 μ M).

Sensory evaluation of prepared functional infused

tea:

Analysis of Variance results indicated no significant difference ($P \leq 0.05$) between the 2 replicates for any of the tested attributes, while there was a significant difference ($P \leq 0.05$) between panelists. Responses of the subjects for color attribute varied from like slightly - extremely like (83.34%) to slightly dislike-dislike extremely (12.5%) while only about 4.17% acted neutral. All panelists accepted the odor with about 92% of responses were like to extremely like compared to about 8% who gave neutral responses. The taste evaluation results showed that 54% of responses were like-extremely like vs. 38% dislike-extremely dislike and about 8% neutral. The clarity attribute score was 71% like-extremely like vs 21% dislike-dislike extremely. The overall acceptability of the tannin-infused tea indicated that 62.5% of the taste panelists liked the product vs. 25% disliked it and 12.5 were neutral. From these scores and accompanying comments (Table 3), it was concluded that tannin-tea can be accepted by the consumers. Moreover, as taste is the most important attribute, it was improved by the addition of cinnamon which increased its acceptability. Clarity, an important attribute in tea and contrary to our expectation was not influenced by the inclusion of the bark powder in the tea.

Conclusions

Oak (*Quercus Aegilops* L.) root bark contains high amounts of tannins mainly as hydrolysable which in turn consist mainly of ellagitannins. The purified tannins possess antioxidant power, as evaluated by their radical scavenging capacity, superior to that of ascorbic acid and have an astringency score similar to tannic acid. Functional tea containing oak root bark powder was accepted by taste panelists which indicates the possible

use of the powder in the preparation of other high-antioxidant functional foods.

Acknowledgments

The authors thank the deanship of scientific research at the University of Jordan for supporting this work which was submitted by author Abdullah in partial fulfillment of the requirements of the Ph.D. degree in food science.

REFERENCES

- Amarowicz R. and Pegg R. (2006). Content of proanthocyanidins in selected plant extracts as determined via n-butanol/HCl hydrolysis and a colorimetric assay or by HPLC. *Polish Journal of Food and Nutrition Sciences*, 15/56/ (3): 319–322.
- Andrenseka S., Simonovska B., Vovka I., Fyhrquist P., Vuorelab H. and Vuorela P. (2004). Antimicrobial and antioxidative enrichment of oak (*Quercus robur*) bark by rotation planar extraction using extra chrome. *International Journal of Food Microbiology*, 92: 181-187.
- Ashok P. and Upadhyaya K. (2012). Tannins are astringent. *Journal of Pharmacognosy and Phytochemistry*, 1: 45-50.
- Bawadi, H. A., Bansode, R. R., Trappey II, A., Truax, R. E., and Losso, J. N. (2005). Inhibition of Caco-2 colon, MCF-7, and Hs578T breast, and DU 145 prostatic cancer cell proliferation by water-soluble black bean condensed tannins. *Cancer letters*, 218(2): 153-162.
- Baxter N., Lilley T., Haslam E. and Williamson M. (1997). Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry (NY)*, 36:5566-5577.
- Boumerfeg, S., Baghiani, A., Djarmouni, M., Ameni, D., Adjadj, M., Belkhiri, F., and Arrar, L. (2018). Inhibitory activity on xanthine oxidase and antioxidant properties of *Teucrium polium* L. extracts. *Chinese Medicine*.3:30-41.
- Chavan U.D., Shahidi F. and Nacz M. (2001). Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents. *Food Chemistry*, 75: 509-512.
- Cobzac S., Moldovan M., Olah N. K., Bobos L. and Surducian E. (2005). Tannin extraction efficiency, from *Rubus idaeus*, *Cydonia oblonga*, and *rumex acetosa*, using different extraction techniques and spectrophotometric quantification. *Seria F. Chemia*, 8: 55-59.
- De Wet H. (2010). Medicinal plants used for the treatment of diarrhea in northern Maputaland, Kwa Zulu-Natal province, South Africa. *Journal of Ethno pharmacology*, 130: 284-289.
- Engström, M., Päljjarvi, M. and Salminen, J. (2015). Rapid fingerprint analysis of plant extracts for ellagitannins, gallic acid, and quinic acid derivatives and quercetin-, kaempferol- and myricetin-based flavonol glycosides by UPLC-QqQ-MS/MS. *Journal of Agricultural and Food Chemistry*, 63(16), 4068-4079
- Falcão L., Araújo M. (2011). Tannin's characterization in new and historic vegetable-tanned leathers fibers by spot tests. *Journal of Cultural Heritage*, 12: 149-156.
- Feng S., Cheng S., Yuan Z., Leitch M. and Xu C. (2013). Valorization Of Bark for Chemicals and Materials: A Review. *Renewable and Sustainable Energy Reviews*,

- 26: 560–578.
- Gangwal A. (2013). Extraction, estimation and thin layer chromatography of tannins: a review. *International Journal of Pharmaceutical and Chemical Sciences*, 2: 1585-1588.
- Glabasnia, A. and Hofmann, T. (2006). Sensory-directed identification of taste-active ellagitannins in American (*Quercus alba* L.) and European oak wood (*Quercus robur* L.) and quantitative analysis in bourbon whiskey and oak-matured red wines. *Journal of Agricultural and Food Chemistry*, 54(9), 3380-3390.
- Gonultas, O. and Ucar, M. (2012), August. Chemical composition of some commercial tannins produced in Turkey. In Proceedings of the 55th International Convention of Society of Wood Science and Technology. Beijing, China: Society of Wood Science and Technology.
- Gonzalez-Castejon M. and Rodriguez-Casado A. (2011). Dietary phytochemicals and their potential effects on obesity: a review. *Pharmacological Research*, 64: 438–455.
- Gülcin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of toxicology*, 86(3), 345-391.
- Guimarães-Beelen P., Berchielli T., Beelen R., Filho J., de Oliveira S. (2006). Characterization of condensed tannins from native legumes of the Brazilian northeastern semi-arid. *Scientia Agricola*, 63: 522-528.
- Hagerman A. (2002). *The tannin handbook, the biological activity of tannins*. Miami University. FL., USA.
- Harbertson J., Parpinello G., Heymann H. and Downey M. (2012). Impact of exogenous tannin additions on wine chemistry and wine sensory character. *Food Chemistry*, 131: 999-1008.
- Hartzfeld P., Forkner R., Hunter M. and Hagerman A. (2002). Determination of hydrolyzable tannins (gallotannins and ellagitannins) after reaction with potassium iodate. *Journal of Agricultural and Food Chemistry*, 50: 1785-1790.
- Hummer W. and Schreier P. (2008). Analysis of proanthocyanidins. *Molecular Nutrition and Food Research*, 52: 1381-1398.
- Karioti, A., Bilia, A. and Skaltsa, H. (2010). *Quercus ilex* L.: a rich source of polyacrylate flavonoid glucosides. *Food Chemistry*, 123: 131–142.
- Khoddami, A., Wilkes, M. and Roberts, T. (2013). Techniques for analysis of plant phenolic compounds. *Molecules*, 18(2), 2328-2375.
- Kumari, M. and Jain, S. (2012). Tannins: an antinutrient with positive effect to manage diabetes. *Research Journal of Recent Sciences*, 1: 70-73.
- Lipińska, L., Klewicka, E. and Sojka, M. (2014). Structure, occurrence and biological activity of ellagitannins: a general review. *ACTA Scientiarum Polonorum Technologia Alimentaria*, 13: 289-299.
- Liu, S. and White, E. (2012): Extraction and characterization of proanthocyanidins from grape seeds. *The Open Food Science Journal*, 6: 5-11.
- Lorrain, B., Ky, I., Pechamat, L. and Teissedre, P. (2013): Evolution of analysis of polyphenols from grapes, wines, and extracts. *Molecules*, 18: 1076-1100.
- Makkar, H. (2000). *Quantification of tannins in tree foliage- a laboratory manual, a Joint FAO/ IAEA working document*, Vienna, Austria, 4-7.
- Makkar, H., Singh, B., Vats, S. and Sood, R. (1993). Total phenols, tannins, and condensed tannins in different parts of *Rumex hastatus*. *Bioresource Technology*, 45: 69-71.
- Mammela, P., Savolainen, H., Lindroos, L., Kangas, J. and Vartiainen, T. (2000). Analysis of oak tannins by liquid chromatography-electrospray ionization mass spectrometry. *Journal of Chromatography*, 891: 75–83.
- Marinho, C., Martucci, M., Gobbo-Neto, L. and Teixeira, S. (2018). Chemical composition and secretion biology of the floral bouquet in legume trees (Fabaceae). *Botanical Journal of the Linnean Society*, 187(1), 5-25.
- Munoz, A. (Ed.). (2013). Sensory evaluation in quality control. *Springer Science & Business Media*.

- Ncube, E., Mhlongo, M., Piater, L., Steenkamp, P., Dubery, I. and Madala, N. (2014). Analyses of chlorogenic acids and related cinnamic acid derivatives from *Nicotiana tabacum* tissues with the aid of UPLC-QTOF-MS/MS-based on the in-source collision-induced dissociation method. *Chemistry Central Journal*, 8(1), 66.
- Padayachee, A., Day, L., Howell, K., and Gidley, M. (2017). Complexity and health functionality of plant cell wall fibers from fruits and vegetables. *Critical Reviews in Food Science and Nutrition*, 57(1), 59-81
- Gülcin, I. (2012). Antioxidant activity of food constituents: an overview. *Archives of Toxicology*, 86(3), 345-391.
- Phakthong, W., Liawruangrath, B. and Liawruangrath S. (2014). Determination of gallic acid with rhodanine by reverse flow injection analysis using simplex optimization. *Talanta*, 130: 577–584
- Quideau, S., Deffieux, D., Douat-Casassus, C. and Pouysegu, L. (2011). Plant polyphenols: chemical properties, biological activities, and synthesis. *Angewandte Chemie International Edition*, 50(3), 586-621.
- Romani, A., Ieri, F., Turchetti, B., Mulinacci, N., Vincieri, F. and Buzzini, P. (2006). Analysis of condensed and hydrolysable tannins from commercial plant extracts. *Journal of Pharmaceutical and Biomedical Analysis*, 41: 415–420.
- Roshni, S. and Ramesh, G. (2013). Preliminary phytochemical screening of *Quercus infectoria* olivier fagaceae. *International Journal of Advances in Pharmacy Biology and Chemistry*, 2: 346-350.
- Sakagami H., Jiang Y., Kusama K., Atsumi T., Ueha T., Toguchi M., Iwakura I., Satoh K., Ito H. Hatano T., and Yoshida, T. (2000): Cytotoxic activity of hydrolyzable tannins against human oral tumor cell lines - a possible mechanism. *Phytomedicine*, 7: 39-47.
- Saad, H., Charrier-El Bouhtoury, F., Pizzi, A., Rode, K., Charrier, B. and Ayed, N. (2012). characterization of pomegranate peels tannin extractives. *Industrial Crops and Products*, 40: 239– 246.
- Sabri, F., Belarbi, M., Sabri, S. and Alsayadi, M. (2012). Phytochemical screening and identification of some compounds from mallow. *Journal of Natural Product and Plant Resources*, 2: 512-516.
- Sánchez-Rangel, J., Benavides, J., Heredia, J., Cisneros-Zevallos, L. and Jacobo-Velázquez, D. (2013). The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. *Analytical Methods*, 5(21), 5990-5999.
- Sáyago-Ayerdi, S., Moreno-Hernández, C., Montalvo-González, E., García-Magaña, M., Mata-Montes de Oca, M., Torres, J. and Pérez-Jiménez, J. (2013). Mexican ‘ataulfo’ mango (*Mangifera Indica* L.) as a source of hydrolyzable tannins. analysis by MALDI-TOF/TOF MS. *Food Research International*, 51: 188–194.
- Shad, M., Nawaz, H., Rehman, T., Ahmad, H., and Hussain, M. (2012). Optimization of an extraction efficiency of tannins from *Cichorium Intybus* L.: application of response surface methodology. *Journal of Medicinal Plants Research*, 28: 4467-4474.
- Sharma, S., Kori, S. and Parmar, A. (2015). Surfactant mediated extraction of total phenolic contents (TPC) and antioxidants from fruits juices. *Food Chemistry*, 185, 284-288.
- Shukla, S., Mehta, A., John, J., Singh, S., Mehta, P. and Vyas, S. (2009). Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. *Food and Chemical Toxicology*, 47: 1848-1851.
- Silanikove, N., Perevolotsky, A. and Provenza, F. 2001. Use of tannin-binding chemicals to assay for tannins and their negative post ingestive effect in ruminants. *Animal Feed Science and Technology*, 91: 69-81.
- Statistical Analysis System Institute9SASI. (2000). *SAS Users Guide in Statistics*, 5th ed. SAS Institute, Cary, NC, USA.
- Zhang, B., Cai, J., Duan, C., Reeves, M., and He, F. (2015). A review of polyphenolics in oak woods. *International Journal of Molecular Sciences*, 16(4), 6978-7014.
- Zhou, H., Lin, Y., Wei, S., Tam, N. (2011). Structural

diversity and antioxidant activity of condensed tannins fractionated from mangosteen pericarp. *Food Chemistry*, 129: 1710-1720.

Appendices

Table 1: Qualitative colorimetric tests of oak root bark methanolic extract and standards¹.

Substrate	Type of Test				
	Ferric Chloride	Vanillin	Acid-butanol	Nitrous acid	Rhodanine
Positive indicates presence of	Phenolics, Gallotannic acid	Condensed tannin	Condensed tannin	Ellagitannins	Gallic acid
Result of methanolic extract	+ Bluish-black	+ Slight brownish red	+ orange-Red	+ Dark blue to purple	+ Pink
Standards					
Tannic acid	Charcoal-black	-	-	-	-
Gallic acid	Black	-	-	-	Pink
Catechin	Black	Red-Crimson	-	Yellow	-
llagic acid	Black	-	-	Red	Yellow
Epicatechin	Black	Red	-	Yellow	-

*+: Positive

*-: Negative

¹ The same results were obtained from the three replicate samples

Table 2: Tannin content of oak root bark expressed as (% w/w)^{1,2}.

total tannins	total CT by two methods		total HT	HT components		
	vanillin (as catechin)	butanol-HCl (as cyanidin eq.)		as tannic acid	free gallic acid	gallo-tannins (as gallic acid)
11.39± 0.95	2.05±0.08	1.64± 0.05	9.02±0.17	0.82± .001	1.88%± 0.06	6.12± 0.22

¹ CT= Condensed Tannins. HT= Hydrolysable Tannins.

² each means is the average of three replicates followed by the standard deviation(± SD).

Table 3: Per cent responses¹ of panelists to the functional tea containing oak root bark powder 2,3.

Characteristics/ Parameter	score									Total %
	1	2	3	4	5	6	7	8	9	
Color	0.0	0.0	8.33±5	4.17±0.3	4.17±0.2	12.5±0.6	41.67±3.5	0.00	29.17±2.5	100
Odor	0.00	0.00	0.00	0.00	8.33±0.4	20.83±2.2	20.83±1.7	29.17±2.5	20.83±1.5	100
Taste	0.00	4.17±0.5	4.17±0.4	29.1±2.5	8.33±0.3	8.33±0.5	8.33±0.6	12.50±1.0	25.00±1.3	100
Clarity	0.00	0.00	20.83±1.5	0.00	8.33±0.5	20.83±1.5	8.33±0.5	8.33±0.5	33.33±2.7	100
Overall Acceptability	4.17±0.3	4.17±0.4	4.17±0.2	12.50±0.8	12.50±0.8	4.17±0.2	25.00±2.8	12.50±0.8	20.83±2.6	100

¹ Percent of 25 responses.

² each value represents mean of 2 readings by the per cent responses ± SD.

³(9) Like extremely, (8) Like very much, (7) Like moderately, (6) Like slightly, (5) Neutral/ neither like nor dislike, (4) Dislike slightly, (3) Dislike moderately, (2) Dislike very much, (1) Dislike extremely.

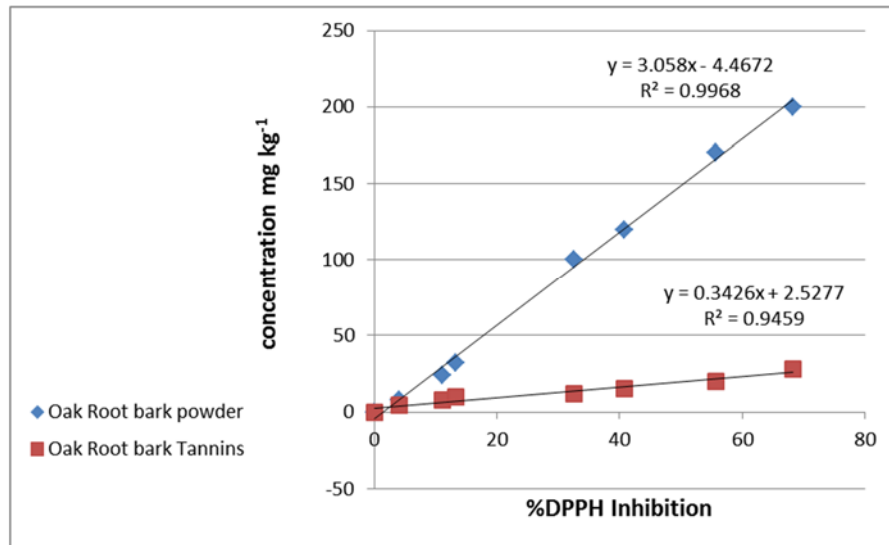


Figure 1: DPPH inhibition (%) effect of the oak root bark powder and its tannins as a function of concentration.

تتينات قلف جذور البلوط *Quercus aegilops* L. ومنع الأكسدة والاستعمال في إعداد شاي وظيفي

عايد شاكر عمرو¹، موسى نعمان أحمد¹، مي عدنان عبد الله²

¹ قسم التغذية والتصنيع الغذائي / كلية الزراعة/ الجامعة الأردنية/ عمان 11942

² قسم التغذية والتصنيع الغذائي/ كلية الحصن الجامعية/ جامعة البلقاء التطبيقية / الحصن/ إربد 21510.

تاريخ استلام البحث 2019/12/23 وتاريخ قبوله 2020/10/12.

ملخص

تم استخلاص مركبات التتین من قلف جذور البلوط في موسمين متعاقبين. وتم تحديد درجة القبوضة والمقدرة على منع الأكسدة للمستخلص باستعمال طريقة الانتشار الشعاعي لاليومين المصل البقري وطريقة DPPH على التوالي. دلت النتائج أن قلف جذر هذا النوع من البلوط يحتوي على 11.39% وزنا من مركبات التتین على شكل حامض الجالیک وأن حوالي 79% منها تتبع مجموعة التتينات القابلة للتحلل HT والباقي بشكل مركبات تتينات مكثفه CT وأن القابلة للتحلل منها تتكون بشكل رئيسي من مركبات الإلاجي تانین وبنسبة أقل من مركبات الجالوتانین. دلت النتائج أيضاً على أن مسحوق الجذور الخام وتتيناته المنقاه لها درجة قبوضة تبلغ 15.8 و 138.9 ملغ البيومين / غم على التوالي وكذلك أن درجة مقدرة المسحوق الخام والتتينات المنتقاة على منع الأكسدة بلغت وفق طريقة ال DPPH ما مقداره 149.7 و 19.24 على التوالي مقارنة بحامض الأسكوربيک ما يدل على تفوق هذه التتينات عليه كموانع للأكسدة. وعندما استعمل مسحوق الجذور في إعداد شاي وظيفي دلت نتائج التقييم الحسي أن الشاي كان مقبولاً للمتذوقين.

الكلمات الدالة: التتينات القابلة للتحلل، التتينات المكثفه، الأغذية الوظيفية.