SHORT REPORT

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Evaluation of Phenolic Contents and Bioactivity of Root and Nutgall Extracts from Iraqian *Quercus infectoria* Olivier

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Abstract: The extracts of powdered root and nutgall of Iraqian Aleppo oak (*Q. infectoria*) were obtained by using three different solvents along with two extraction methods. Liquid chromatography and tandem mass spectrometry (LC-MS/MS) was implemented to identify phytochemicals in the extracts. Antioxidant activity was determined by DPPH radical scavenging activity. Also, for measurement of antibacterial activity, disc diffusion and microdilution assays were used. Specifically, the nutgall extracts were found to have higher concentration of phenolic acid contents, and to some extent flavonoids and greater antioxidant and antimicrobial activities in comparison with the root extracts. Furthermore, microwave extraction technique was proven to be much more effective than conventional one in view of extraction yield for both plant parts used here.

Keywords: *Quercus infectoria*; LC-MS/MS; Antioxidant activity; Antimicrobial activity.

1. Plant Source

Oak trees (genus Quercus and Family Fagaceae) vary from small bushes to great trees and are attended essentially in the North Temperate Zone, growing in different of habitats such as mountain slopes and wet lowlands [1]. It is less abundant and deciduous species; restricted largely indigenous in Turkey, Iran, Iraq, Cyprus, East Aegean Islands, Greece, Lebanon and Syria. Plant parts used of *Q. infectoria* for medicinal properties are mainly root, stem or bark, leaf, valonia-type fruit, seed and nut/apple galls [2].

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2. Previous Studies

The main components found in *Q. infectoria* are tannins, polyphenols, sugar, starch and essential oils. Also, it may be a good source of minerals to treat a number of diseases [3]. Pharmacologically, it was reported that *Q. infectoria* exhibited astringent, wound healing, anti-inflammatory, antiviral, larvicidal, antibacterial, antiulcerogenic and gastroprotective effects [1]. However, none of the study had reported on the root extract to identify and quantify of phytochemicals, antioxidant and antimicrobial activities of *Q. infectoria*.

3. Present Study

To identify and quantify of phytochemicals in the root and nutgall extracts of *Q. infectoria* LC-MS/MS were used. For evaluation of antioxidant and antimicrobial activities of this plant, DPPH scavenging capacity and disc diffusion methods were respectively used.

The extraction yield was strictly dependent on the nature of extracting solvents and methods due to the presence of different availability of bioactive components, resulting from the varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. Methanol extracted the most components from the plant, followed by ethanol and water, respectively. The extraction yield of the plant depicted that polar compounds in biological herb were easier to extract with polar solvents. Based upon the LC-MS/MS fingerprints, it could be concluded that this analytical technique is a modern method to diagnose the presence of numerous constituents present in the extract of *Q. infectoria* [4]. Validation and uncertainty parameters for phenolic compounds as well as quantification of the methanol extracts of root and nutgall from the *Q. infectoria* were given in Table 1. Furthermore, the LC-MS/MS chromatograms for 27 phenolic reference compounds used for calibration and validation were illustrated in Figure 1 and Table 1.

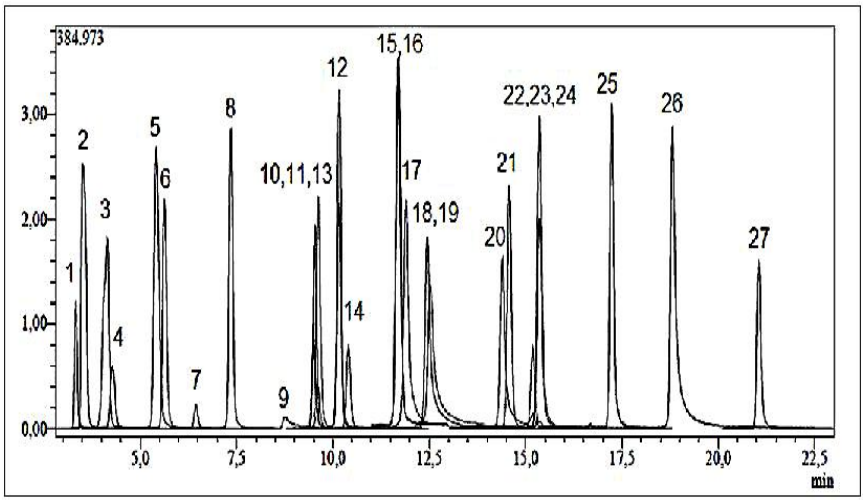

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Figure 1. LC-MS/MS chromatogram for reference phenolic compounds: 1) quinic acid, 2) malic acid, 3) tr-aconitic acid, 4) gallic acid, 5) chlorogenic acid, 6) protocatechuic acid, 7) tannic acid, 8) tr-caffeic acid, 9) vanillin, 10) p-coumaric acid, 11) rosmarinic acid, 12) rutin, 13) hesperidin, 14) hyperoside, 15) 4-OH benzoic acid, 16) salicylic acid, 17) myricetin, 18)

fisetin, 19) coumarin, 20) quercetin, 21) naringenin, 22) hesperetin, 23) luteolin, 24) kaempferol, 25) apigenin, 26) rhamnetin, and 27) chrysin.

Table 1. Phytochemical parameters and phenolic quantifications of the methanol extract of root and nutgall from *Q. infectoria* determined by LC-MS/MS chromatography

No	Analytes	RT[1]	Parent ion (m/z)[2]	Linear Range (mg/L)	LOD/LOQ (µg/L)[3]	Recovery (%)	U_{95} (%)[4]	Quantification[5] µg/g (w/w)	
								Root	Nutgall
1	Quinic acid	3.32	190.95	250-10000	22.3 / 74.5	103.3	4.8	2766	8672.7
2	Malic acid	3.54	133.05	250-10000	19.2 / 64.1	101.4	5.3	845.5	2167.9
3	tr-Aconitic acid	4.13	172.85	250-10000	15.6 / 51.9	102.8	4.9	4.701	25.382
4	Gallic acid	4.29	169.05	25-1000	4.8 / 15.9	102.3	5.1	77.25	3724.12
5	Chlorogenic acid	5.43	353	250-10000	7.3 / 24.3	99.7	4.9	0.204	19.025
6	Protocatechuic acid	5.63	152.95	100-4000	25.8 / 85.9	100.2	5.1	3.525	119.084
7	Tannic acid	6.46	182.95	100-4000	10.2 / 34.2	97.8	5.1	1128	91422.9
8	tr-Caffeic acid	7.37	178.95	25-1000	4.4 / 14.7	98.6	5.2	0.378	10.0584
9	Vanillin	8.77	151.05	250-10000	10.1 / 33.7	99.2	4.9	3.172	17.7008
10	*p*-Coumaric acid	9.53	162.95	100-4000	15.2 / 50.8	98.4	5.1	3.617	5.1362
11	Rosmarinic acid	9.57	358.9	250-10000	10.4 / 34.8	101.7	4.9	0.122	0.0438
12	Rutin	10.18	609.1	250-10000	17.0 / 56.6	102.2	5.0	0.149	2.4745
13	Hesperidin	9.69	611.1	250-10000	21.6 / 71.9	100.2	4.9	0.299	24.788
14	Hyperoside	10.43	463.1	100-4000	12.4 / 41.4	98.5	4.9	7.296	44.534
15	4-OH Benzoic acid	11.72	136.95	25-1000	3.0 / 10.0	106.2	5.2	0.874	7.5236
16	Salicylic acid	11.72	136.95	25-1000	4 / 13.3	106.2	5.0	0.886	6.6071
17	Myricetin	11.94	317	100-4000	9.9 / 32.9	106.0	5.9	0.511	0.54704
18	Fisetin	12.61	284.95	100-4000	10.7 / 35.6	96.9	5.5	0.098	0.00957
19	Coumarin	12.52	146.95	100-4000	9.1 / 30.4	104.4	4.9	0.203	0.164
20	Quercetin	14.48	300.9	25-1000	2.0 / 6.8	98.9	7.1	0.034	3.7597
21	Naringenin	14.66	270.95	25-1000	2.6 / 8.8	97.0	5.5	0.052	0.110
22	Hesperetin	15.29	300.95	25-1000	3.3/ 11.0	102.4	5.3	0.036	0.0374
23	Luteolin	15.43	284.95	25-1000	5.8 / 19.4	105.4	6.9	0.138	0.1357
24	Kaempferol	15.43	284.95	25-1000	2.0 / 6.6	99.1	5.2	0.051	0.6318
25	Apigenin	17.31	268.95	25-1000	0.1 / 0.3	98.9	5.3	0.011	0.0701
26	Rhamnetin	18.94	314.95	25-1000	0.2 / 0.7	100.8	6.1	0.163	0.0639
27	Chrysin	21.18	253	25-1000	0.05 / 0.17	102.2	5.3	N.D.[5]	N.D.[5]

[1]RT: retention time, [2] Parent ion (m/z): molecular ions of the standard compounds (mass to charge ratio), [3]LOD/LOQ: Limit of detection/Limit of quantification, [4]U_{95}: percent relative uncertainty at 95% confidence level (k:2), and [5] N.D.: Not detected. [5]Solvent: methanol; Extraction method: Microwave-assisted extraction technique.

The overall results were rich in terms of phenolic and non-phenolic compounds. It is also evident from Table 1 that the nutgall extracts had much higher concentration of phenolic contents, including phenolic acids and flavonoids, in comparison with the root extracts. On the other hand, all extracts studied herein showed quite higher concentrations of phenolic and non-phenolic acids compared with those of flavonoids. The nutgall extracts comprised, mainly, of tannic, quinic, gallic, malic and protocatechuic acids, respectively. Moreover, they contained small amounts of various flavonoids such as hyperoside, hesperidin, quercetin and rutin as well as some other flavonoids. However, the root extracts were found to consist, essentially, of non-phenolic acids such as quinic, malic and gallic acids, consequently, along with small amounts of various flavonoids. The analysis revealed that phenolic acids were a major phytochemical in the

whole extracts obtained herein. Our finding was similar to the results observed by Kaur et al. [5], who studied that the extracts from the root and nutgall of *Q. infectoria* contained a huge amount of polyphenols and had a potent reducing power. When compared to literature, this study was the first one, which reports such a high amount of polyphenols in the root extract of *Q. infectoria.*

For determination of antioxidant activity of the root and nutgall extracts from *Q. infectoria* DPPH scavenging assay was used [6,7]. Table 2 lists the DPPH scavenging capacity of the root and nutgall extracts from *Q. infectoria* and control as functions of two different extraction techniques and solvents. As shown in Table 1, based on the results obtained from *Q. infectoria*, the methanol extracts of the gall with higher amount of phenolics had a remarkable effect on radical scavenging activities, meaning that methanol was more effective solvent compared to the other solvents used wherein. In the meantime, the microwave-assisted extraction (MAE) exhibited somehow greater DPPH radical scavenging activity when compared with the conventional extraction technique (CET) for both extracts. However, the extracts were better DPPH radical scavenging activity than the butylated hydroxytoluene (BHT), synthetic antioxidant and positive control, at the same conditions. In the present study, the polyphenols contributed significantly to the antioxidant activity and acted as greatly effective free radical scavengers which are mainly due to their redox properties, which could play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides [8]. Similarly, Gülçin *et al.* [9] showed the correlation between phenolics found in extracts. Kaur *et al*. [5] found that ethanolic extract of *Q. infectoria* galls entirely scavenged DPPH radicals at low concentration (1.5 μg/mL).

Table 2. DPPH radical scavenging capacity of the root and nutgall extracts from *Q. infectoria* and control as functions of two different extraction techniques and solvents

Fraction*	DPPH Scavenging Capacity (%)					
	Microwave Assisted Extraction			Conventional Extraction Technique		
	Methanol	**Ethanol**	**Aqueous**	**Methanol**	**Ethanol**	**Aqueous**
Root	95±0.43	94±0.40	39±1.99	94±0.50	93±0.98	34±1.25
Nutgall	96±0.04	95±0.90	52±0.69	95±0.03	94±0.23	44±0.69
BHT	94±0.50	78±0.22	18±0.82	94±0.50	78±0.22	18±0.82

*****Each fraction expressed as mean ± S.D. Samples were analyzed in triplicate.

The microdilution results of *Q. infectoria* were in the agreement with the disc diffusion assays through various plant parts extracted by different solvents and methods [10]. For determination of MICs of *Q. infectoria* extracts 96-well plates were used. This event might be explained by the amount of bioactive compounds and their diffusivity in the growth media. As depicted in Table 3, the nutgall extracts were determined to have higher inhibition zone and much lower MIC values when compared with the root extracts for all the microorganisms used here. It was interesting to notice that the extracts obtained by using the microwave-assisted extraction method had higher antimicrobial activity rather than the extracts from the conventional extraction one. This phenomenon might be ascribed to higher concentration of the phenolics provided by using the MAE technique. It was also proven that the whole nutgall extracts obtained in this study demonstrated greater antimicrobial activities against all the microorganisms employed here in comparison with control bactericide and fungicide. However, the root extracts showed almost similar antimicrobial activity tendencies to the controls used here. Like other natural products, the bioactive compounds in the extracts from *Q. infectoria* against microorganisms inhibited cell wall synthesis, accumulated in microbial membranes causing energy depletions, or interfered the permeability of cell membranes, consequence made increase in permeability, loss of cellular constitutes, and membrane disruption, and modified the structure and function of key cellular constituents, resulting in mutation, cell damage, and death [11].

On the other hand, the gram-positive bacterial strains were more susceptible than the gram-negative bacteria owing to cell wall components and its thickness. However, fungi were strongly influenced by a mean inhibition zone of *Q. infectoria* extracts. This result was very interesting since the fungi had been the most extensively studied pathogen in antifungal resistance due to their morbidity and mortality associated with infections in immune compromised patient. The investigation indicated good ability of the extracts to be sensitive for microorganisms as compared to the standard drugs.

Table 3. Antimicrobial activity (mm) and MIC (mg/mL) parameters of the root and nutgall extracts of *Q. infectoria* along with control.

Extracts		Microwave Assisted Extraction						Conventional Extraction Technique						Control
		Methanol		Ethanol		Aqueous		Methanol		Ethanol		Aqueous		
		AA	MIC	AA	MIC	AA	MIC	AA	MIC	AA	MIC	AA	MIC	
Root	Bs	16 ±0.20	6.25	15 ±0.47	4.166	12 ±0.50	6.25	17 ±0.00	3.125	15 ±0.47	6.25	13 ±2.00	4.166	16 ±1.10
	Bm	15 ±0.57	3.125	14 ±0.00	4.166	13 ±0.73	4.166	14 ±0.00	6.25	13 ±0.47	4.166	14 ±1.24	6.25	15 ±0.09
	Sa	17 ±0.63	4.166	14 ±2.00	3.125	13 ±0.47	12.5	17 ±0.25	3.125	17 ±0.00	3.125	13 ±0.47	6.25	20 ±1.71
	Ec	15 ±0.57	6.25	13 ±1.24	6.25	12 ±0.81	6.25	13 ±0.47	6.25	15 ±0.47	6.25	12 ±1.41	6.25	13 ±1.71
	Pa	17 ±0.47	6.25	15 ±2.00	6.25	14 ±0.47	6.25	13 ±1.63	6.25	15 ±0.00	6.25	14 ±0.47	6.25	16 ±0.94
	Kp	14 ±1.00	4.166	14 ±0.81	6.25	13 ±0.81	12.5	15 ±1.63	4.166	14 ±0.47	6.25	13 ±0.00	6.25	14 ±0.63
	Ca	16 ±0.40	4.166	14 ±0.81	6.25	12 ±1.24	6.25	16 ±2.00	6.25	15 ±1.63	6.25	12 ±1.24	12.5	20 ±0.71
	Yl	13 ±0.00	6.25	11 ±0.94	6.25	00 ±0.00	--	13 ±0.00	6.25	11 ±0.47	6.25	00 ±0.00	--	11 ±0.44
Nutgall	Bs	24 ±0.57	2.083	23 ±1.40	4.166	19 ±1.24	4.166	24 ±0.47	6.25	22 ±0.47	4.166	19 ±1.24	4.166	16 ±1.10
	Bm	25 ±0.57	3.125	22 ±0.33	4.166	21 ±0.81	4.166	22 ±0.81	2.083	20 ±1.24	3.125	18 ±0.47	6.25	15 ±0.09
	Sa	29 ±0.47	2.083	25 ±2.00	3.125	22 ±1.24	4.166	27 ±0.32	2.083	22 ±0.24	3.125	20 ±0.24	4.166	20 ±1.71
	Ec	24 ±1.00	2.083	23 ±0.00	3.125	20 ±0.47	6.25	23 ±1.24	4.166	22 ±0.81	4.166	19 ±0.81	6.25	13 ±1.71
	Pa	23 ±0.47	3.125	21 ±0.00	6.25	18 ±0.81	6.25	22 ±0.81	4.166	20 ±1.24	4.166	18 ±0.00	4.166	16 ±0.94
	Kp	24 ±1.00	3.125	23 ±0.25	3.125	20 ±1.24	6.25	21 ±1.63	3.125	21 ±0.47	3.125	20 ±0.47	4.166	14 ±0.63
	Ca	21 ±1.63	4.166	19 ±0.47	4.166	18 ±0.47	4.166	19 ±0.28	6.25	17 ±0.25	4.166	15 ±0.47	6.25	20 ±0.71
	Yl	22 ±0.22	4.166	18 ±0.81	6.25	15 ±0.00	6.25	18 ±0.81	4.166	15 ±1.63	4.166	13 ±0.47	6.25	11 ±0.44

AA: Antimicrobial activity (disc diffusion assay), **MIC:** Minimum Inhibitory Concentration (Microdiluton assay) Bacteria: **Bs:** *B. subtilis*, **Bm:** *B. megaterium*, **Sa:** *S. aureus*, **Ec:** *E. coli*, **Pa:** *P. aeruginosa*, **Kp:** *K. Pneumonia*, **Ca:** *C. albicans*, **Yl:** *Y. lipolytica*. Each fraction expressed as mean ± S.D. Samples were analyzed in triplicate. **Control:** Gentamycin (20 µg) for bacteria and Flagyl (25 µg) for fungi.

In addition, *Q. infectoria* extracts were found to have pronounced inhibitory effect at low concentration of MIC. This result was very interesting because the possible toxic effects of active compound might be minimized when used in very low concentration (Table 3). Consequently, *Q. infectoria* showed a broad-spectrum agent, which could be used against gram positive, gram-negative bacteria and also fungi. Similarly, it was reported on the minimum inhibitory properties of medicinal plants against a various pathologic sources [12,13]. Overall, we suggested that the extracts of this plant was broad spectrum in their activities so that the bioactive compounds had a great significance in therapeutic treatments.

Basri and Fan [14] reported that the aqueous and acetone extracts from the galls of *Q. infectoria* showed similar effects in antimicrobial activities and had MIC value ranging between 0.0781 mg/mL and 1.25 mg/mL, which were lower than the values found here. Furthermore, the antibacterial activities of essential oil, ethanolic and aqueous extracts of leaves from *Q. infectoria* against some important food borne bacteria were also studied [15]. They found that the extracts had a good enough antimicrobial activity against food borne pathogens, revealing that they could be used in food preservation systems to block the growth of these bacteria and to increase food quality and safety.

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