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CHEMICAL COMPOSITION OF ESSENTIAL OIL BY SPME AND EVALUATION OF ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES OF *Quercus inectoria* GALL

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ABSTRACT

Quercus infectoria galls have been utilized to cure widespread illnesses. In line with it, this paper aims to investigate the chemical combination of the volatile oils obtained from galls of *Q. infectoria* and evaluate their antioxidant and antimicrobial features. The volatiles of *Q. infectoria* has been isolated by solid-phase microextraction (SPME). The compositions of the volatiles were revealed via gas chromatography coupled with mass spectrometry (GC-MS). Twenty-nine substances have been characterized in the volatile essential oil of *Q. infectoria*. The major components of the volatile oil were (Z)-Anethol 28.55%, pentadecanolide (26.44%), diethyl phthalate (6.46%), and acetoin (5.66%). *Q. infectoria* showed good-moderate antimicrobial (*Staphylococcus aureus*, *Streptomyces griseolus*, *Pseudomonas citronellosis*) and antifungal (*Candida glabrata*, *Candida krusei*) activities against to test microorganisms with MIC value 250 µg/mL and 125 µg/mL, respectively. Also, total flavonoid and total polyphenol amounts were found for *Q. infectoria,* and our total polyphenol result (342.87 mg GAE/g) was found higher than the studies in the literature. At the same time *Q. infectoria* has been observed to have high antioxidant activity according to DPPH, FRAP, and CUPRAC assays. Especially it exhibited excellent DPPH activity for the IC_{50} rate of 0.002 µg/mL which is higher than the standard Trolox (IC_{50} = 0.008 µg/mL). This study is important because it is the first one, which reports the determination of essential oil, total polyphenol, flavonoid contents, antioxidant, and antimicrobial activities all together for *Q. infectoria.*

Key words: *Quercus infectoria*, GC-MS, SPME, volatile oil, antioxidant, antimicrobial

INTRODUCTION

Food-borne pathogenic is a community of microorganisms that reason food-borne illness. Food-borne illnesses occurring because of the consumption of nutrient contaminated with pathogenic microorganisms are a large issue in public health and has become a great reason of death rate in persons with disrupted immune systems in developing countries [Nabi et al. 2014]. In the last centenary, the plentiful utilize of antibiotics has caused the appearance of a series of medicine-resistant bacteria, yeast, fungi, and parasite [Gholamreza et al. 2013]. Observing antimicrobial resistance through improving antibiotic use and

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reducing hospital infection is therefore an urgent requirement. However, new antibiotics (natural) should continue to be developed in order to maintain the effect of primary antibacterial therapy [Lund and O'Brien 2011, Tajkarimi et al. 2010].

Oxidative harm to cubicles is thought to be a very significant agent in different clinical diseases and in the ordinary stage of aging [Halliwell et al. 1992]. Many illnesses like cancer, diabetes, heart disease, and others are caused by oxidative stress, and it can cause free radicals in nutrition, medicines, and living organisms [Young and Woodside 2001]. Intake of antioxidants can avert or impede oxidative damage [Robak and Marcinkiewicz 1995]. Synthetical antioxidants like butyl hydroxytoluene (BHT) and butylhydroxyanisole (BHA) are present but they have potency health hazards and toxicity [Madhavi and Salunkhe 1995]. Therefore, these necessities to be replaced with natural antioxidants. Medicinal plants used in traditional medicine and healing are among natural antioxidant sources. Plants are rich sources of phenoliccompounds such asflavonoids, tannins and anthocyanins, which are the most important natural antioxidant [Kamkar et al. 2013].

Due to customers who fear the harms of chemical preservatives, the demand for natural biological compounds has increased, and a new trend has emerged in the food industry for alternative approaches to food preservation (green consumerism). Essential oils and extracts from natural additives have a high value for use in the green consumer market due to their biological activities [Homayonpour et al. 2021].

Actually, throughout the last decades, attention in the utilize of herbal products has risen the use of plants [Meléndez and Capriles 2006]. Plants play a major part in health. Likewise, it is forecasted that 25% of contemporary medicine were obtained directly or indirectly via plants [Tambekar and Dahikar 2011]. Many plants are available in the literature as compounds with antifungal, antiparasitic, antiviral, and antioxidants effects [Kordali et al. 2005, Lucera et al. 2012]. Medicinal plants utilized in conventional drugs appear to be a wealthy resource of native and safe biocidal metabolites. Essential oils produced from these herbs and their ingredients represent a resource of native antimicrobial and antioxidant materials and have the potency to be utilized in the nutrient industry to rise the rack life of food products without any side effects [Tajkarimi et al. 2010, Solorzano-Santos and Mmiranda-Novales 2012]. Aromatic volatile oils are frequently utilized as a flavoring and prevent the expansion of mildew and bacterial contaminants in the nutrient industry [Lopes-Lutz et al. 2008].

Oak trees with the scholarly noun of *Quercus*, a small tree or bush of about to 4 to 6 feet tall that grows in Asia Minor, Turkey, Greece, and Iran, is one of the popular medicinal plants which has been conventionally utilized [Dar et al. 1976, Jamzad et al. 2012]. *Quercus* is a plant genus in the family of Fagaceae which has more than 45 species, among which *Q. infectoria* is highly distributed in Kurdistan and West Azerbaijan mountains. *Q. infectoria* is a tree that grows wild in abandoned areas such as Turkey, Syria, Iran, and Iraq [Shrestha et al. 2014]. This species is generally known under the name baloot in Iran and are commonly used as a medicinal plant [Fathabad et al. 2015].

Diverse pieces of oak are known to possess multiple medicinal properties and are utilized widely in a few folk medicines as an antiparkinsonian, analgesic CNS depressant, anti-inflammatory, and anti-diabetic medicine [Iminjan et al. 2014]. Furthermore, in conventional medicine, *Q. infectoria* is utilized in the therapy of eczema, intertrigo, chronic diarrhoea, hemorrhages, dysentery, and impetigo [Basri and Fan 2005].

The Quercus species generate a globally known fruit (acorn) and, together with bark and leaves, has been utilized in conventional medicine, applied as in gastrointestinal or antiseptic disorders. Due to their nutritional property, acorns are utilized as food for both humans and animals. At the same time, the diversity of folk uses and the richness of phytochemicals found in Quercus species make this genus interesting for assessing biological activities and toxicological effects [Burlacu et al. 2020].

The major components found in *Q. infectoria* are polyphenols, sugar, starch, tannins, and essential oils [Sucilathangam et al. 2012]. On the other hand, some factors like plant kinds and pieces, cultivar sex, geographical root, harvesting period, and climatic terms could affect the ingredients and functional property of herbs [Saedi et al. 2015].

The importance of antioxidant compounds derived from plant material in health and protection against disease is confirmed with related studies [Iminjan et al.

2014, Burlacu et al. 2020]. Utilizing *Q. infectoria* extracts, an essential oil in nutraceuticals, nutrient preparations, or cosmetic anti-aging formularization can be promising for health. Hence the aim of this paper was to investigate volatiles compounds by solid-phase microextraction (SPME) and assess the antioxidant and antimicrobial properties of the extracts obtained from *Q. infectoria* galls that have been traditionally used as general health supplements. There are no prior reports on the determination of essential oil contents, antioxidant, and antimicrobial activities of *Q. infectoria* galls all together. Therefore, this study is the first to evaluate all these features together.

MATERIALS AND METHODS

The chemicals. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), methanol, 2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-1picrylhydrazyl (DPPH), and Folin-Ciocalteu's phenol agent were bought from Sigma Chemical Co. (St. Louis, MO, USA). Neocuproine (2,9–dimethyl-1,10-phenanthroline), Na_2CO_3 (sodium carbonate), CH_3COOH (acetic acid), $Al(NO_3)_3.9H_2O$ (aluminum nitrate nonahydrate), and $C_2H_7NO_2$ (ammonium acetate) were obtained from Merck Chemical Co. (Darmstadt, Germany). Substances were at the analytic degree.

The plant material. Dry galls of *Q. infectoria* utilized in this paper were obtained from the regional market (herbalist) of Mardin province. The plant is edible and has been utilized as a medical plant by local folk for many years in Mardin. The galls were crushed to little parts utilizing pestle and mortar and powdered in an electric grinder. About 10 g of dry sample was weighed to prepare the methanolic extract, then it was stirred at room temperature for 24 hours (one day) with a magnetic mixer. Prepared methanolic solutions were filtered with a filter paper [Ceylan et al. 2009]. This herb extract was utilized to obtain antioxidant properties. Analyzes were repeated three times. And spectrophotometric methods were utilized on antioxidant analyses, total flavonoids, and total polyphenols. Spectrophotometric techniques are often utilized for the standardization of native crude substances. In addition, antimicrobial activity and essential oils of the plant were determined.

Identification of the essential oil components

Procedure of solid phase microextraction (SPME). The dry plant material, *Q. infectoria* (5 g), was crushed into little pieces and added in a sealed bottle (10 mL) with a silicone-caoutchouc septum flap, then the headspace was placed to the solid--phase microextraction apparatus (Supelco, USA). A DVB/Carboxene/PDMS coating fiber was added to the headspace and utilized to get volatile substances. The SPME fibers were situated for 5 min at 250°C in the GC injector. Extraction was succeeded with magnetic stirring at 80°C utilizing an incubation for 5 min and an extraction for 10 min. Fiber with extract of volatile components was later on injected into the GC injector. The transporter gas was helium at a flux ratio of 1 mL/min. The injection was completed in divide mode (1 : 30) at 250°C. The sample was analyzed and reported. The temperature, extraction, and incubation period time adjusted according to the analyzed and reported test [Renda et al. 2016].

Gas chromatography-mass spectrometry (GC/MS). Gas chromatography-flame ionization detector (GC) analysis was performed on a Shimadzu QP2010 plus gas chromatography utilizing a TRB-5MS capillary column (30 m × 0.25 mm, film thickness, 0.25 μm). Shimadzu QP2010 Plus gas chromatography was connected to a Shimadzu QP2010 Ultra mass selector detector. The split mode was used, and the split amount was 1 : 30. The bakery program was as follows: the first heat was 40°C for 2 minutes, which was raised to 240°C in 3 minutes, the last heat was kept at 250°C for 4 minutes, and the total analysis period time was 55 minutes. The mass transfer line and injector heats were set at 200°C and 250°C, respectively. The utilized transporter gas was Helium (99.999%) with a stable flux amount of 1 mL/minute. Detection was performed in the electronic pulse range (EI). Ionization tension adjusted to 70 eV, and scanning range (40–400 m/z) was utilized to get mass [Renda et al. 2016].

Identification ofconstituents. Retention index of overall compounds was obtained by the Kovats technique utilizing C7-C30 alkane standards [Kovats 1958]. The constituents of the oils were determined via comparison of their mass spectrum with those of mass spectrum FNSC 1.3 library [FFNSC 2008].

Total phenolic assay. The total phenolic amount of herbs was obtained by utilizing the Folin-Ciocal-

teu assessment [Slinkard and Singleton 1977]. Standard was gallic acid (1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 mg/mL) in this work. Shortly, 0.5 N and 400 µL Folin-Ciocalteu tests, 20 µL methanolic samples (1 mg/mL), 680 µL of distilled water, and 20 µL of gallic acid (diverse concentrations) were stirred. Then the solution was stirred. After 3-minute waiting, 400 µL of Na_2CO_3 (10%) mixture was annexed and again vortexed. Then the solution waited for nearly 2 h. After that, absorbances of the solutions were determined for 760 nm. Total phenolic compounds from concentrations were determined for a dry weight of a sample as mg of gallic acid.

Total flavonoid assay. The amount was determined by utilizing aluminum chloride analysis [Chang et al. 2002]. Standard was Quercetin. 0.1 mL 1 M NH_4CH_3COO, 4.3 mL methanol, 0.5 mL of Quercetin (0.03125; 0.0625; 0.125; 0.25; 0.5 and 1mg/mL), and 0.1 mL 10% Al $(NO_3)_3$ were put in the tubes and then they were vortexed. Mixtures was left for about 40 minutes. Absorbances were determined for 415 nm. Flavonoid amounts for herbs were defined for the dry weight of a sample as mg quercetin.

The determination of antioxidant activity. The antioxidant contents of the plants were calculated utilizing FRAP and CUPRAC techniques. FRAP test was utilized to calculated antioxidant activity. The technique is a reduction of Fe^{3+}-TPTZ compound to the Fe^{2+}-TPTZ compound with electron giving material to this situation [Benzie and Szeto 1999]. The 100 µL of the test extract or the blank and 3 mL of FRAP test (including TPTZ, $FeCl_3$, and acetate buffer) were annexed and vortexed. The absorbance rates on 593 nm were determined at 25°C for about 4 min. The standard graph for 100–1000 µmol/L was compared with the last absorbance. The result was explained for a dry sample as µmol $FeSO_4.7H_2O$.

The CUPRAC technique consists of measuring the absorbance of this mixture at 450 nm 60 minutes after mixing the antioxidant mixture and aqueous ammonium acetate (pH 7), a neocuproin alcoholic solution and copper (II) chloride solution [Apak et al. 2004]. 1mL NH_4Ac (1M), 1mL Neocuproine (7.5 mM), and 1mL $CuCl_2$ (10) mM were annexed, after that a sample with 0.9 mL H_2O and 0.2 mL was annexed and vortexed. The latest amount was 4.1 mL. Later, the last absorbance value was calculated at 450 nm. Conclusions were appraised by Trolox ® equivalent antioxidant activity (TEAC).

The radical cleaning capacity of samples of DPPH radical (2,2-diphenyl-1-picrylhydrazyl) was defined utilizing the technique of Molyneux [Molyneux 2004]. Various concentrations of 0.75 ml of each sample extract were vortexed together with 0.75 ml of a 0.1 mM of DPPH solution in methanol. After that, each extract was left at room heat in the dark (50 min).

Absorbance was determined via a spectrophotometer (517 nm). Trolox is utilized as stock, and amounts are explained as IC_{50} (mg sample per mL).

The biological materials. A sum of 13 microorganisms was utilized in this paper. As bacteria, *Escherichia coli* ATCC 8739, *Bacillus velezensis* NRRL B-14580, *Proteus vulgaris* NRRL B-123, *Salmonella typhimurium* ATCC 13311, *Gordonia rubripertincta* NRRL B-3906, *Staphylococcus epidermidis* ATCC 12228, *Streptomyces griseolus* NRRL B-1062, *Bacillus subtilis* NRRL B-4378, *Staphylococcus aureus* ATCC 6538, *Pseudomonas citronellosis* NRRL B-2504, as yeast; *Candida krusei* ATCC 6258, *Candida albicans* ATCC 90028, *Candida glabrata* ATCC 2001, strains were used. All test microorganisms were got from the United States Agricultural Research Service Culture Collection (NRRL), the American Type Culture Collection (ATCC), the Faculty of Pharmacy of Anadolu University, and the commercial culture collections.

All microorganisms were placed at –85°C (Ultrafreezer, NewaBrunswick) in 15% glycerol and maintained to make nutrient agar (Merck, 1.05450) and malt extract agar (Merck, 1.05398) slants at 4°C, respectively. They were subcultured in Petri materials before being utilized for the purity control.

Microorganisms, which are significant herb and person pathogens, were chosen in different studies for their antibacterial property which give biofilms and have been the topic of the study done by numerous scientists in last works.

***In vitro* antimicrobial activity.** The broth microdilution technique suggested by CLSI (Clinical Laboratory Standards Institute) was utilized for assessing *in vitro* antifungal and antimicrobial properties of substances [Amsterdam 1996]. Tetracycline and chloramphenicol were utilized as stock antimicrobial materials whereas ketoconazole and amphotericin B were uti-

lized as stock antifungal materials. They were bought from Sigma. All trials were analyzed in duplicate in two independent tests.

Broth microdilution test for bacteria. Broth microdilution trial was conducted according to the principles of the CLSI M100-S16 [CLSI 2006]. The minimum inhibitory concentration (MIC) of the galls of *Q. infectoria* was analyzed by broth microdilution technique utilizing 96-well microtiter plates (Sigma, Germany). Microbial suspensions have grown overnight in double strength Mueller-Hinton broth (MHB) (Merck, Germany) they were standardized metrically to about 10^8 CFU 1/mL (using Mac Farland No: 0.5) with turbido. Test components were solved in DMSO (10%) and diluted in MHB to get a concentration value of 15.63–4000 µg / mL. DMSO was utilized as a negative check. The solution was then diluted two times in MHB (100 L), vaccinated with microbial strains, and then left at 37°C for 24 hours. Resazurin (Sigma, Germany) solution was annexed to verify MICs. The MIC endpoint was described as the minimum concentration with full (100%) growth inhibition. The outcomes of antibacterial trials were compared with those of stocks chloramphenicol (Sigma, Germany) and ampicillin (Sigma, Germany) like antimicrobial materials (The last concentrations were range 0.25 and 32 µg/mL). DMSO was evaluated as a negative check.

Broth microdilution test for yeasts. CLSI Broth microdilution trialing was also conducted completely as stated in paper M27-A2 by utilizing 96-well microtiter plates, RPMI-1640 (Sigma, Germany) medium, and inocula of 0.5–2.5×103 cells /mL (Mac Farland 0.5). The last concentrations of the galls of *Q. infectoria* were in the range of 15.63 and 4000 µg/mL. MIC ranges were obtained for 24 h at 37°C incubation. Resazurin solution was annexed to approve the MICs. The MIC endpoint was described as the minimum concentration with full (100%) growth inhibition [CLSI 2002].

RESULTS

Chemical composition. The results of solid phase microextraction and gas chromatographic analysis connected with mass spectrometry GC-MS of the essential oils of the *Q. infectoria* are given in Table 1. A total of 29 components were determined and quantified from the gall species. Chemical ingredients were described on the foundation of a characteristic library research and literature datum [24]. (Z)-Anethol and pentadecanolide were the major compounds of the gall species. Acetoin was the first starting compound appearing in the column (RT = 1.624 min), while (E) -Tridec-2-enal was the longest held compound in the column (RT = 51.412 min). According to results, (Z)-Anethol is the major constituent of this essential oil of *Q. infectoria* with a content of about 28.55%, followed by pentadecanolide (26.44%), diethyl phthalate (6.46%), and acetoin (5.66%). The other constituents are detected as an average percentage: menthalactone (2.15%), 2-ethylhexanol (1.43%), piperitone (1.42%), 16-hydroxy-6-hexadecenoic acid omega lactone (1.48%), pelargonaldehyde (1.10%), and ethylene brassylate (1.22%). The content of the rest of the constituents is often less than 1%. The compounds were separated into 11 classes, which were monoterpenoids, sesquiterpene, sesquiterpenoid, aldehydes, ketones, alcohols, fatty acids, ether, esters, hydrocarbons, and others (Tab. 1).

Antioxidant activity. In this paper, chiefly three techniques, DPPH radical scavenging activity, CUPRAC (cupric reducing power), FRAP (ferric reducing power) were utilized to determine the antioxidant property of *Q. infectoria*. The contents of total flavonoids and phenolic were also described for the extracts. The total flavonoid and phenolic content, DPPH, CUPRAC, and FRAP values were summarized in Table 2. The results shown in the tables refer to the average ±SD of three parallel measurements. The IC_{50} values found from a test of DPPH were showed in Fig. 1. IC_{50} value was calculated from the linear regression analysis (Microsoft Excel, Microsoft Corporation®, USA). Outcomes showed that *Q. infectoria* galls were obtained to have a high total flavonoid and phenolic amount. In addition, *Q. infectoria* galls extract fully scavenged DPPH radicals at an excessively low concentration with the IC_{50} value of 0.002 µg/mL.

Antimicrobial activity. The antimicrobial property of the solution of *Q. infectoria* analyzed toward the microorganisms in this paper was quantitatively and qualitatively stated by appraising the presence of MIC values, zone diameter, and inhibition zones. The antimicrobial property of the solution was tested against 10 bacteria and 3 yeasts. The microorganisms chosen for the antibacterial property studies are between sig-

Table 1. Identified Components in the Essential oils of *Q. infectoria*

No	Compounds	RT (min)	Area%	Exp. [a]RI
	Monoterpenoids			
1	Isoisopulegol	17.964	0.72	1180
2	Carvone	20.463	0.53	1248
3	Piperitone	20.843	1.42	1267
		Total	2.67	
	Sesquiterpene			
4	Beta-chamigrene	28.580	0.40	1486
	Sesquiterpenoid			
5	Viridiflorol	49.063	0.40	2260
	Aldehydes			
6	Pelargonaldehyde	15.240	1.10	1108
7	Capraldehyde	19.070	0.46	1210
8	Caprylaldehyde	46.827	0.40	2162
9	Hendecanal	49.895	0.72	2298
10	Capronaldehyde	50.775	0.41	2339
11	(E)-Tridec-2-enal	51.412	0.76	2369
		Total	4.4	
	Ketones			
12	Acetoin	1.624	5.66	650
13	Menthalactone	27.336	2.15	1448
14	Ethylene brassylate	33.335	1.22	1642
15	Epsilon-decalactone	46.785	0.49	2160
		Total	9.52	
	Alcohols			
16	2-Ethylhexanol	12.391	1.43	1034
17	Benzyl alcohol	12.525	0.39	1038
18	Pelargol	17.831	0.41	1177
		Total	2.23	
	Fatty Acids			
19	Valeric acid	10.551	0.49	987
20	Pelargonic acid	21.389	0.40	1289
21	Palmitic acid	42.157	0.88	1948
22	16-hydroxy-6-hexadecenoic acid omega lactone	45.475	1.48	2103
		Total	3.25	
	Ether			
23	(Z)-Anethol	21.975	28.55	1290
	Esters			
24	Diethyl phthalate	32.151	6.46	1601
25	Methyl palmitate	41.321	0.72	1524
26	Pentadecanolide	46.486	26.44	2147
27	(E)-Citronellyl tiglate	46.745	0.72	2158
		Total	35.29	
	Hydrocarbon			
28	Hexadecane	32.282	0.58	1606
	Others			
29	Unknowns	17.369–50.717	0.41–1.85	–
		Total	12.71	
	Total percentages		100	

[a]RI calculated from retention times relative to that of alkanes (C7-C30) on the non-polar TRB-5MS column. Bold values represent the major components

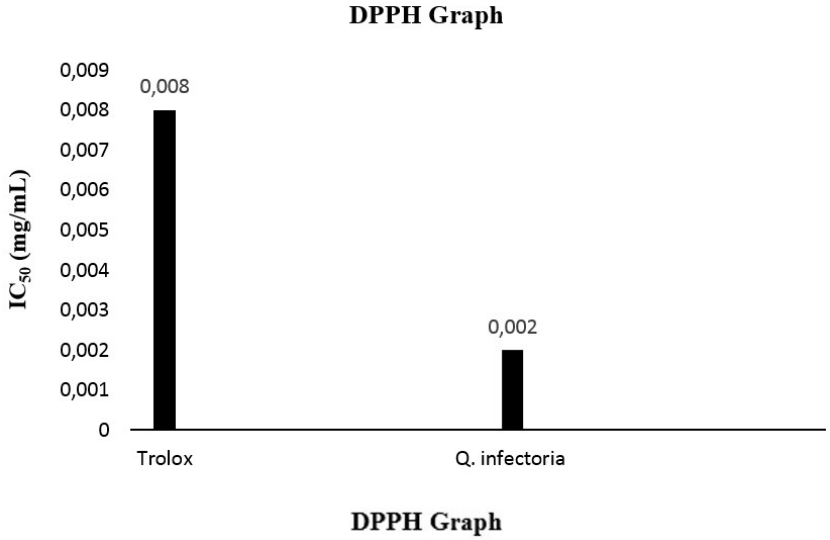

Fig. 1. The result of DPPH for *Q. infectoria* plant extract

Table 2. Results of total phenolic and flavonoid contents, FRAP and CUPRAC values for plant sample

Sample	Total phenolics (mg GAE/g)	Total flavonoid (mg QE/g)	CUPRAC (mmol TEAC/g)	FRAP (µmol Fe/g)
Q. infectoria	342.87 ±20.34	0.75 ±0.07	13.23 ±0.51	4.04 ±0.21

Table 3. MIC values of *Q. infectoria* against the bacterial strains tested

Samples	Minimal inhibition concentration values (µg/mL)												
	Sa	Pv	St	Se	Bs	Sg	Pc	Bv	Gr	Ec	Ca	Cg	Ck
Q. infectoria	250	500	2000	1000	4000	250	250	1000	2000	4000	250	125	125
Chlor.	0.5	0.5	0.125	1	0.125	0.125	1	4	0.125	0.25			
Tet.	0.125	8	0.125	8	0.125	0.125	0.25	4	0.125	1			
AmfB.											0.25	0.125	0.125
Ket.											0.062	0.062	0.125

Sa: *Staphylococcus aureus* ATCC 6538, Pv: *Proteus vulgaris* NRRL B-123, St: *Salmonella typhimurium* ATCC 13311, Se: *Staphylococcus epidermidis* ATCC 12228, Bs: *Bacillus subtilis* NRRL B-4378, Sg: *Streptomyces griseolus* NRRL B-1062, Pc: *Pseudomonas citronellosis* NRRL B-2504, Bv: *Bacillus velezensis* NRRL B-14580, Gr: *Gordonia rubripertincta* NRRL B-3906, Ec: *Escherichia coli* ATCC 8739, as yeast; Ca: *Candida albicans* ATCC 90028, Cg: *Candida glabrata* ATCC 2001, Ck: *Candida krusei* ATCC 6258, Chlor: chloramphenicol, Tet: tetracycline, AmfB: amphotericin B, Ket: ketoconazole

nificant herb and man pathogens and biofilm giving microorganisms. These microorganisms have been targeted by many scientists in their recent studies. Table 3 indicates the MIC values of Q. infectoria broth microdilution for isolates. At the concentrations tested, *Q. infectoria* shows an exhibited good antifungal activity (125 µg/mL) against *Candida glabrata* and *Candida krusei*. Also, the plant shows antibacterial activities against *Staphylococcus aureus*, *Streptomyces griseolus*, and *Pseudomonas citronellosis* (250 µg/mL).

DISCUSSION

In literature surveys, different large components were obtained in GC-MS assays of the galls of *Q. infectoria* for the plant growing in Iraq [Hussein et al. 2016, Hamad et al. 2017]. All chemical structure of the volatile oils demonstrated a lot of differences as in our situation, which can be described by the subspecies of the herb utilized, locality, harvest time, and environment. Also, there are not many studies in the literature on the essential oils of the galls of *Q. infectoria.* When compared to literature, this paper is the first one, which reports the determination of essential oil contents, antioxidant, and antimicrobial activities, all together. That's why it is very important to investigate the new biological features of this gall of *Q. infectoria* which has many medicinal properties [Dar et al. 1976, Jamzad et al. 2012, Iminjan et al. 2014, Basri and Fan 2005, Ahmad et al. 2011] and add these activities into the literature.

Results showed that *Q. infectoria* galls were observed to have a high total flavonoid and phenolic amount. The study has been conducted on the *Q. infectoria* grown in Iran [Kheirandish et al. 2016]. Total flavonoid and polyphenol amounts have been determined as 57.50 and 1.86, respectively. Yet, according to our study, total flavonoid and polyphenol amounts were found to be 342.87 and 0.75. Our total polyphenol result was found higher than the studies in the literature. It is well-known that, in plants, the antioxidant property is directly concerned with the level of phenol and flavonoid components. The key act of phenol components as the eliminator of free radicals has been offered by different studies [Katalinic et al. 2006, Theriault et al. 2006].

According to the results of the CUPRAC method, antioxidant activity was observed at 13.23 ±0.51 mmol TEAC/g. Additionally, according to the results of the FRAP method, antioxidant activity was found 4.04 ±0.21 µmol Fe/g. While the DPPH activity determination method, which is one of the antioxidant content determination methods for the *Q. infectoria* plant, is available in the literature, there are no studies on the CUPRAC and FRAP methods. This paper was the first one, which reports the determination of the antioxidant activity of *Q. infectoria* for CUPRAC and FRAP methods.

Antioxidants decrease DPPH, a purple-painted steady free radical, to an uncolored α-α-diphenyl--β-picrylahydrazine. Scavenging of DPPH radicals is a powerful vehicle to learn the antioxidant property of a product. Thus, the antioxidant activity assay was conducted to investigate the ability of *Q. infectoria* to scavenge free radicals in vitro. *Q. infectoria* galls extract fully scavenged DPPH radicals at a highly low concentration with the IC_{50} value of 0.002 µg/mL. It was even found to have a lower concentration than Trolox (IC_{50} = 0.008 µg/mL) used as a standard in the DPPH analysis. In addition, *Q. infectoria* galls have more DPPH activity than standard Trolox. Also, the DPPH activity result obtained in this study is better than the DPPH results found for this *Q. infectoria* plant in the literature [Iylia Arina and Harisun 2019, Kaur et al. 2008, Hasmida et al. 2014].

Studies on the antimicrobial activity of the Q. infectoria plant are also available in the literature and are in line with our results. *Q. infectoria* galls demonstrated a broad-spectrum material, which could be utilized towards gram-negative, gram-positive bacteria, and also fungi [Hamad et al. 2017, Basri and Fan 2005, Leela and Satirapipathkul 2011].

There are many antibacterial herbs, some of which have been discovered and some of which are unknown in nature. So, recently has been a rise in the study of plants as a medical resource. Volatile oils and extracts are potency resources of new antibacterial substances, particularly against microbial pathogens. These native components may also be applied for the cure of infective illnesses, thus it is efficient as antibacterial property [Sandri et al. 2007, Torbati et al. 2013].

CONCLUSIONS

This paper determined the chemical structure of the volatile oils got from galls of *Q. infectoria* and evaluated their antimicrobial and antioxidant activities. As can be seen from our results, the essential oils of *Q. infectoria* contain many main ingredients in large amounts and high antioxidant, antifungal activity. Especially *Q. infectoria* gall extract was found to show an excellent DPPH antioxidant activity with the IC_{50} value of 0.002 μg/mL. It was even found to have a lower concentration than Trolox (IC_{50} = 0.008 μg/mL) used as a standard in DPPH analysis. That is *Q. infectoria* galls have more DPPH activity than standard Trolox. To summarize, we have shown that *Q. infectoria* extract can be considered as an antioxidant. The replacement of synthetic with natural antioxidants may be advantageous. Therefore, these essential oils can be improved and utilized in food supplements to fulfill the need and can also be utilized as natural resources in pharmaceutical/chemical/cosmetic sectors for their antioxidant, antifungal, and antibacterial properties. At the same time, this study sheds light on future in vivo studies to demonstrate the bioactivity properties and toxic effects of *Q. infectoria*. The pharmacological researches have mostly been performed in vitro and in vivo, and clinical researches are very limited. More studies are needed to show the link between the chemical compound and bioactivity and to discuss their action mechanism. These studies should be constantly developed using newer techniques.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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