# Phytochemical Profile and Antifungal Effect of (*Quercus infectoria* Oliv.) Plant Root Extract on Several Candida spices

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**Abstract**

**Objective:** Biological effect of the antifungal activity, antioxidant activity, total tannin estimation and phytochemical profile determination of extract.

**Methodology:** The root extracted was investigated as antifungal activity against seven types of Candida spp. such *as Candida albicans, Candida glabatra, Candida Krusei, Candida tropicalis, Candida famata, Candida parapsilosis* and *Candida guilliermondii* each paper disk 6mm was contained (50µL) of extract. Conventional method and ethanol solvent used for extraction.

**Results:** It was documented that plant extracts have inhibitory effects against seven types of tested candida with the observations that the root extract the lowest inhibitory zone 15.33mm of extract at was against *Candida tropicalis*. Whereas, the highest inhibitory zone 21.26mm was against *Candida albicans*. The yield was found (14.51%). Total condensed tannin was viewed (2.24 mg/kg). The highest scavenging activity was 0.1 ml (99.57%). The non-phenolic compounds high amount was quinic acid (10,110.50µg/g). The non-flavonoids high amount was gallic acid (1,182.93µg/g). The maximum of flavonoids compounds was rutin (41.70µg/g). Which was used LC–MS/MS for analysis.

**Conclusion**

In a conclusion, this research exposed that the extracts can posses' antioxidant activity and comparable to those got from commercial antioxidant. The non-phenolic acid, non-flavonoid and flavonoids are current in the root extract. The strong antioxidant and antifungal activities of extracts are probably due to the presence of the bioactive compound in the plants. Additionally, the consequences exposed big sources of quinic acid, gallic acid and rutin in Aleppo Oak root which might give an original source of this biological antifungal activity and antioxidant.

*keywords: Candida spp, Antifungal, Antioxidant, Aleppo Oak, the phenolic compound, condense tannin.*

## Introduction

Plants have been the main source of medicines since ancient times. Practically all human cultures have been used plants not only as nutrition sources but also as healing against ailment and diseases[1].

Medicinal plants which are extracts and used indirectly or directly as a treatment for diverse ailments. Today, most developing countries used medicinal plant and traditional medicine as a basis for good health, has been widely observed. In the world, nearly 30 % of the therapeutic are preparations from medicinal plants[2].

Plants that have been used for the old-style drug include a huge quantity of materials applicable for the dealing of chronic illnesses and the variety of infections. The substances accomplished by inhibiting the infectious development or killing them are considered as promising candidates for the cure of numerous infectious sicknesses. As a traditional remedy, therapeutic plants are well distinguished in rural communities of many growing countries [3][4].

Applying plant extracts and phytochemical combined with antimicrobial properties can be of great implication in therapeutic actions. In latest years, many types research has been shown around the world to prove such effectiveness. Numerous plants have been applied in view of their antimicrobial behaviours which are because of compounds synthesized in the plant secondary metabolism [5].

The body of human resistance system with its enzymatic and non-enzymatic antioxidant systems can limit and defend the body against reactive oxygen species (ROS) [6]. However, the natural defense may not be sufficient for severe or continued oxidative stress [7]. So, certain quantities of exogenous antioxidants are continually required to preserve an adequate level of antioxidants to re-equilibrium the ROS in the human body. Several synthetic antioxidants, such as Butylated Hydroxyanisole (BHA) extremely effective. However, owing to side impacts of synthetic antioxidants, the request for natural antioxidants has been increased [8].

Among the 50 000 to 250 000 species of fungi that have been described, fewer than 500 have been associated with human disease, and no more than 100 are capable of causing infection in otherwise typical individuals. In general, these organisms are free-living in nature and are in no way dependent on humans (or animals) for their survival [9][10].

Tannins have antifungal action [11]. At current, the medicinal physiological activities of tannins are one of the warm spots in the topic of natural products. The multiple functions and applications of plant tannins make us see a broader perspective, which also presses us to make a more effective in-depth research and development on the basis of existing knowledge [12].

Candida species are the microorganisms normal flora, which is isolated from the human body and two trimmer pathogens, they able to cause diverse clinical appearances of candidiasis [13].

Phenolics are categorized by having a minimum one aromatic ring with one or more hydroxyl groups adhered. In extra of 8000 phenolic structures have been appeared and they are extensively scattered throughout the kingdom of plants [14]. Phenolics array from unpretentious, low molecular-weight, only aromatic-ringed complexes with complex and large tannins and derived polyphenols. They can be arranged based on the amount and the array of their carbon atoms and are usually found integrate organic acids and sugars. Phenolics can be divided into two groups: the non-flavonoids and the flavonoids [15].

The objective of this study was the antioxidant and antifungal activities of Aleppo Oak root plant comparison with six artificial antifungals. Then, total tannin and yield estimated. Finally, it was described the phytochemical profile of root ethanol extract by using LC-ESI-MS/MS.

## Material and Methods

### Preparation of plant root sample

The herbarium code of Aleppo Oak (*Quercus infectoria* Oliv.) was (7463). The new sample was washed with the distilled water to remove the undesired particles and the greatest

important thing to do with freshly collected material to dry it as fast as possible to avoid fungal infection and preserve color. At that time, the plant had been dried at room temperature. Next, the dried sample was crushed into powder (less than 2mm) by using the new modern grinder YAZICILAR (Model G1) and stored at 2- 4 °C in dark containers [16].

**Extract preparation**

Ten grams of sample powder was mixed with 100 ml of solvents and added into around bottom flask. The ratio of plant material mass (g) to solvent volume (ml) was 1:10. The conventional extraction was performed at boiling point temperature for 60 minutes. Further, the temperature was standard for Ethanol 68ºC. Then, the mixtures were allowed to cool at room temperature. Next, the mixture was filtered and evaporated in a rotary vacuum evaporator so that obtain a purified extract [17].

**Determination of extraction yield**

The extraction yield is a measure of solvent efficiency to extract specific components from the original material and it was defined as the quantity of extract recovered in mass compared with the initial amount of dry sample [18]. Powder of root (0.5g) was extracted with 50 ml ethanol distinctly by using extraction method described before. The yield percentage of the extract was determined by using the following formula:

$$\text{Yield percentage (\%)} = \frac{X}{Y} \times 100$$

where,
X is the oven dry weight of extract (g),
Y is the oven dry weight of the sample (g).

**Determination of total condensed tannin**

Extraction solution was ready by mixing 0.05g of $FeSO_4$, 95 ml N-butanol and 5 ml Hydrochloric acid (35%). For determining the condensed tannin, 0.01g of plant root pawder and mimosa tannin put separately in a test tube and 10 ml of extraction solution was added and placed in the water bath for heating 1h. The absorbance was measured at the 580nm wavelength. This analyze was carried out by UV/Vis spectrophotometer (Shimadzu PC-1280). [19].

**DPPH radical scavenging activity**

The plant extract of free radical scavenging activity of root had been concluded by means of the DPPH assay explained by the moderate alteration [20]. In its radical form, 1,1-diphenyl-2picrylhydrazyl (DPPH) absorption level decreases at 517nm with reduction of an antioxidant or a radical compound. Briefly, 0.1mM DPPH was organized for plant extraction. Then 0.1, 0.2 and 0.3 ml of pattern solutions combined with solvent up to 3 ml in a test tube, one by one. Subsequent, 1 ml of DPPH was added. The mixture was then shaken dynamically by Vortex and placed in a dark place at room temperature for 30 min. Later, Shimadzu UV-vis 1240 spectrophotometer at 517nm used for calculating the absorbance of samples. Butylated hydroxytoluene (BHT) was used as a reference. Radical scavenging effectiveness

was expressed as the inhibition percent of free radical through the pattern and calculated with the following equation: [21].

$$\text{DPPH radical scavenging activity (\%)} = \frac{\text{Abs}_{Control} - \text{Abs}_{Test}}{\text{Abs}_{Control}} \times 100$$

Where/
$\text{Abs}_{Control}$ = the absorbance of DPPH radical + Solvent
$\text{Abs}_{Test}$ = the absorbance of the Samples and BHT, separately.

## Phytochemicals analysis

### MS instrumentation

MS detection was achieved using Shimadzu LC-MS 8040 model triple quadrupole mass spectrometer prepared with an ESI source operating in both positive and negative ionization modes. LC-MS/MS data were collected and processed by Lab Solutions software (Shimadzu, Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analyses: the assay of investigated compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and/or the third one for confirmation.

### Method validation parameters for LC-MS/MS

In this investigation, twenty-four phenolic and three non-phenolic organic acids which were prevalent in plant resources were quantified and qualified in plants. Straight-lined regression equations and the linearity ranges of the investigated standard compounds are assumed in Figure 3 [22]. Correlation coefficients were originating to be upper than 0.99. The limit of detection (LOD) and limit of quantitation (LOQ) of their ported analytical method were shown with results in Table 5. For the analyzed compounds, LOD ranged between 0.05 and 25.8 g/L and LOQ ranged between 0.17 and 85.9 g/L. Additionally, the recoveries of the phenolic compounds ranged from 96.9% to 106.2%. The results were calculated by the equation below:

$$\text{Quantification } (\mu\text{g analyte/g extract}) = \frac{Y * U_{95}}{100}$$

Where/
Y: LC-MS/MS results of analyte
U: uncertainty confidence level

## Microorganisms

Completely candida species had been got from (Media Diagnostic Centre) MDC Erbil accredited from America, CAP* (College of American Pathology) so these strains are external quality control material from CAP except *Candida albicans* which is ATCC strain (ATCC 10,231). *Candida albicans* (ATCC 10231), *Candida glabrata, Candida Krusei, Candida tropicalis, Candida famata, Candida parapsilosis* and *Candida guilliermondii*. However, to extend the spectrum of organisms investigated, laboratory strains were also included in the study. Indeed, six types of artificial antifungal discs used such as Nystatin 100unit (NYS), Clotrimazole 10mcg CLT, Fluconazole 25mcg (FLU), Ketoconazole 10mcg

(KTZ), Fluconazole 1010mcg (FLC) and Miconazole 10mcg (MCZ) for comparison with plant antifungal (50 μg/ml).

**Biological evaluates**

The Disc diffusion method was known as the Kirby- Bauer method has been carefully standardized by the Clinical Laboratory Standard Institute (CLSI 2006) methodology. Suspensions of the test organisms were prepared in sterile saline the optical density (OD) of a 0.5 McFarland standard using a densitometer (Turbidity meter BioMerieux, France) [23]. A sterilized cotton swab was dipped into the suspension and inoculated evenly above the whole superficies of the medium by rotating the plate. The discs were 6 mm in diameter contain (50μl) concentration of extracts. Which was applied to the plates individually by using sterile forceps and then gently pressed down onto the agar medium. Usually, no more than 6 disks we are placed on a plate for avoids overlapping of the zones of inhibition and possible error in measurement. Then, for more confirmation by sterile forceps were utilized to place the antibiotic disc on the surface of the inoculums. After the disks were placed on the plate, the plate was inverted and incubated at 37°C (Air incubator, LTE Scientific, UK) for 24h to 48h dependent on strains being tested. Later, the inhibition zone formed by candida inhibition growth. At that moment, it was calculated the inhibition zone by using an electronic ruler. Lastly, each inhibition zone interpreted chart table which is recommended by NCCLS (National Committee for Clinical Laboratory Standards) for antimicrobial susceptibility to testing and monitoring [23].

**The minimum inhibitory concentration determination**

The minimum inhibitory concentration (MIC) values obtained by broth microdilution, for extract activity upon *Candida spp.* were relatively low. It was put 0.9 ml of Sabouraud dextrose broth (SDB) medium. Next, 0.1 ml of extract at concentration 0.1 mg/ml solvent was mixed with broth in serial decimal dilutions diluting 1 ml in 9 ml of broth to get the concentration range of 0.1 to 500 mcg/ml. The inoculum may also be standardized based on optical density [OD625 of 0.08-0.1(1cm light path)] by using a spectrophotometer. This is usually achieved after 18- 24 hours. All the candida suspensions were prepared by suspending 24h *candida* culture in sterile normal saline (0.89% NaCl wt/vol). The turbidity of the candida suspension was adjusted to the optical density (OD) of a 0.5 McFarland standard (equivalent to 1.5 × 108 CFU/ml) [24] [25].

**Statistical analysis**

The data was analysed by ANOVA using GraphPad (Prism 6) statistical program. A statistical analysis Probability value (P-value) less than (<0.05) was considered as statistically significant (*), while P-value more than (>0.05) was considered as statistically not significant. Histogram and pie-chart were used for the statistical analysis of the results.

**Results**

**Antifungal tests**

The root extract was statically significant by the different diameters of inhibition zones of most the activities against the organisms tested were given in Table 1. The mean inhibition

zones against all *Candida* test ranged from 15.3–21.26 mm. The minimum inhibitory zone 15.33 mm of extract at was against *Candida Krusei.* Whereas, the highest inhibitory zone 21.26 mm was against *Candida glabatra.* Indeed, the minimum inhibitory concentration (MIC) values against all *Candida* test was between 7.8125-15.625µg/ml.

The numerous synthetic antifungal was statically significant by the diverse widths of inhibition zones of maximum the activities against the organisms tested were given in Table 1. The mean inhibition zones synthetic antifungal against all Candida test ranged from 10.5–38.40 mm. The minimum inhibitory zone 10.5 mm of FLU/25 was against *Candida albicans.* The uppermost inhibitory zone of FLU/25 38.40 mm was against *Candida guilliermondii*.

**Table 1. Inhibition zones (I.Z.mm) and minimum inhibitory concentration (MIC) of the *Quercus infectoria* ethanol root extracts and synthetic antifungal activities against the *Candida spp*.**

| Candida spp. | | CE4 (I.Z. mm) | MIC (µg/ml) | NY 100U | CLT 10mcg | FLU 25mcg | KTC 10mcg | FLU 10mcg | MCZ 10mcg |
|---|---|---|---|---|---|---|---|---|---|
| *Candida albicans* | Mean | 19.4 | 15.625 | 20.21 | 18.15 | 10.55 | 14.05 | 0.00 | 14.61 |
| | SD | 0.02 | | 0.24 | 0.18 | 0.13 | 0.10 | 0.00 | 0.10 |
| | SE | 0.01 | | 0.10 | 0.07 | 0.05 | 0.04 | 0.00 | 0.04 |
| *Candida glabrata* | Mean | 21.26 | 7.8125 | 24.72 | 12.29 | 15.68 | 13.33 | 16.71 | 14.54 |
| | SD | 0.04 | | 0.31 | 0.22 | 0.21 | 0.11 | 0.16 | 0.20 |
| | SE | 0.02 | | 0.12 | 0.09 | 0.09 | 0.04 | 0.06 | 0.08 |
| *Candida krusei* | Mean | 15.3 | 15.625 | 14.49 | 25.91 | 24.63 | 18.79 | 15.32 | 13.52 |
| | SD | 0.02 | - | 0.17 | 0.22 | 0.66 | 0.09 | 0.15 | 0.20 |
| | SE | 0.01 | | 0.07 | 0.09 | 0.27 | 0.04 | 0.06 | 0.08 |
| *Candida tropicalis* | Mean | 16.42 | 15.625 | 21.23 | 19.95 | 27.67 | 23.96 | 20.52 | 12.46 |
| | SD | 0.01 | | 0.15 | 0.14 | 0.17 | 0.10 | 0.08 | 0.27 |
| | SE | 0.00 | | 0.06 | 0.06 | 0.07 | 0.04 | 0.03 | 0.11 |
| *Candida famata* | Mean | 15.06 | 15.625 | 24.75 | 20.26 | 0.00 | 0.00 | 0.00 | 0.00 |
| | SD | 0.04 | | 0.13 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| | SE | 0.01 | | 0.05 | 0.07 | 0.00 | 0.00 | 0.00 | 0.00 |
| *Candida parapsilosis* | Mean | 17.88 | 15.625 | 24.47 | 22.42 | 26.36 | 27.48 | 21.43 | 11.53 |
| | SD | 0.02 | | 0.15 | 0.28 | 0.17 | 0.32 | 0.16 | 0.17 |
| | SE | 0.01 | | 0.06 | 0.11 | 0.07 | 0.13 | 0.07 | 0.07 |
| *Candida guilliermondii* | Mean | 20.51 | 7.8125 | 21.45 | 31.26 | 38.07 | 37.61 | 37.48 | 24.19 |
| | SD | 0.05 | | 0.18 | 0.19 | 1.21 | 0.32 | 0.26 | 0.15 |
| | SE | 0.02 | | 0.07 | 0.08 | 0.49 | 0.13 | 0.11 | 0.06 |

**Determination of extraction yield of**

The extraction yield in root extract of *Quercus infectoria* prepared by conventional method. The uppermost extraction yield was found in conventional ethanol (14.51%) was observed in Table 2.

**Table 2. The percentage yield of *Quercus infectoria* root extracts**

| | R 1 | R 2 | R 3 | Yield (%) |
|---|---|---|---|---|
| Mean | 14.51 ± | 14.52 ± | 14.50 ± | 14.51 ± |
| SD | 0.061 | 0.006 | 0.005 | |
| SE | 0.021 | 0.002 | 0.003 | |

**Determination total condensed tannin**

Tannin was calculated by the *n*-butanol-HCl-iron way. The use of this assessment for amount of tannin was quantitatively released from the sample through the assess which is showed in Table 3. The greatest commonly used standard for *n*-butanol /HCl assay is mimosa-tannin under normal reaction/condition which calculated using the regression equation ($y = 66.357_x + 0.4117$) got earlier from the linear calibration curve (Figure 3). Comparison the high amount of tannins found in all root plants species by percentage of tannin was determined,

Calculation: % = A/3m,

Where A = absorbance value,

m = mass weight [26].

In this study, tannin-content was diverse decidedly and significantly so that the result was expressed as absorbance unit at 580 $nm_s$ per 1 mg of extract ($A_{580}$/mg). The result was viewed (2.86 mg/kg) as shown in Table 4.

**Table 3. Total condensed tannin**

| | R 1 | R 1 | R 3 | Mean |
|---|---|---|---|---|
| Extract mg/kg | 2.85 | 2.85 | 2.88 | 2.86 |
| SD | 0.15 | 0.13 | 0.013 | |
| SE | 0.06 | 0.05 | 0.04 | |

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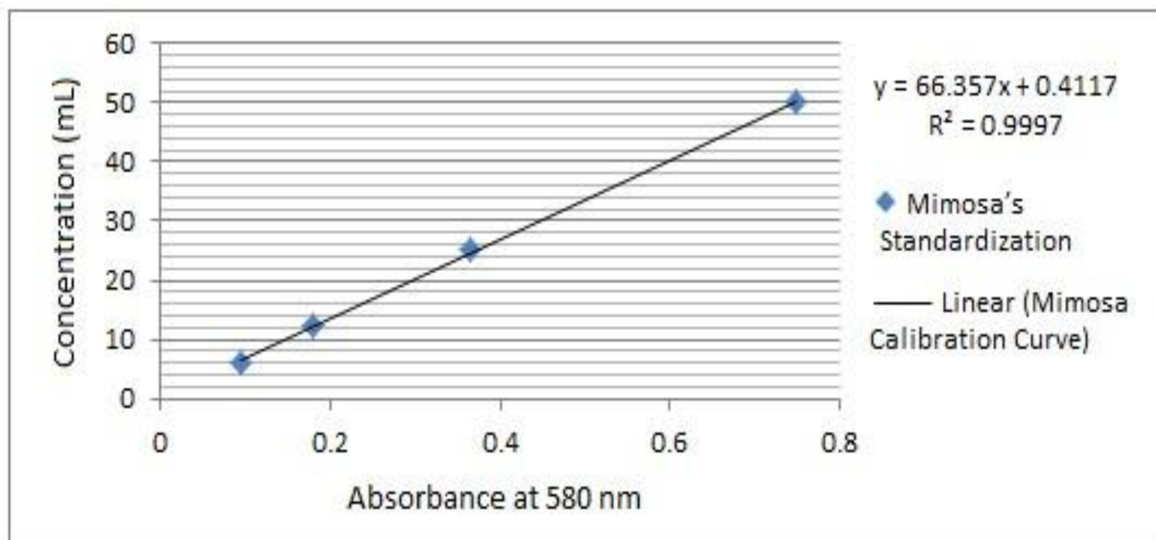


**Figure 1. Mimosa tannin calibration curve**

## Determine the antioxidant activity

The root of *Q. infectoria* extract showed a DPPH radical scavenging activity. The highest scavenging activity at 0.1 mL was (99.57%). While the minimum amount conventional at 0.2 mL was (85.15). The present data's in Table 4 shows the extract was better antioxidants than the controls: BHT (synthetic antioxidant as the positive control) under the same conditions.

**Table 4. DPPH Radical Scavenging activity (%)**

| | MWE 0.1ml | MWE 0.2ml | MWE 0.3ml | BHT 0.1ml | BHT 0.2ml | BHT 0.3ml |
|---|---|---|---|---|---|---|
| Mean % | 99.57 | 85.15 | 93.84 | 65.39 | 65.10 | 71.97 |
| SD | 0.17 | 0.50 | 0.41 | 0.24 | 0.31 | 0.39 |
| SE | 0.07 | 0.20 | 0.17 | 0.10 | 0.13 | 0.16 |

## Analytical parameters

In this investigation, the quantification and determination of phenolic acids in *Quercus infectoria* were carried out by UHPLC-ESI-MS/MS. Many phytochemicals in ethanol root extract were detected. Around investigation, twenty-seven standard kinds have been using three non-phenolic organic acids, eleven phenolic acids and thirteen flavonoid compounds of root ethanol extract.

Primarily, the result was indicated that non-phenolic compounds are abundant in ethanol root extract of *Quercus infectoria*. It was contained a high amount of non-phenolic was quinic acid (10,110.50µg/g). The minimum amount was tr-aconitic acid (43.25µg/g). Also, the high amount of phenolic acids was gallic acid (1,182.93µg/g) and low amount was tr-caffeic acid (6.51µg/g). No detected any rosmarinic acid and coumarin. Throughout our study, thirteen kinds of the flavonoids standard has been used by LC-MS/MS. The maximum and the

minimum of flavonoids compounds were rutin (41.70µg/g) and apigenin (0.09µg/g). Nevertheless, hesperidin, hyperoside, fisetin, quercetin, kaempferol and chrysin were no detected in the ethanol root extract as shown in Table 5 and Figure 2 and 3.

**Table 5 Analytical parameters, identification and quantification of phenolic compounds of ethanol extract of *Quercus infectoria* Oliv by LC ESI MS/MS.**

| No | Analytes | RT[a] | Parent ion (m/z)[b] | $R^{2c}$ | RSD %[d] | LOD/LOQ (µg/L)[e] | Recovery (%) | U[f] | Quantification (µg /g)[g] MWE |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Quinic acid | 3.32 | 190.95 | 0.9927 | 0.0388 | 22.3 / 74.5 | 103.3 | 4.8 | 10110.50 |
| 2 | Malic acid | 3.54 | 133.05 | 0.9975 | 0.1214 | 19.2 / 64.1 | 101.4 | 5.3 | 5530.25 |
| 3 | tr-Aconitic acid | 4.13 | 172.85 | 0.9933 | 0.3908 | 15.6 / 51.9 | 102.8 | 4.9 | 43.25 |
| 4 | Gallic acid | 4.29 | 169.05 | 0.9901 | 0.4734 | 4.8 / 15.9 | 102.3 | 5.1 | 1182.93 |
| 5 | Chlorogenic acid | 5.43 | 353 | 0.9932 | 0.1882 | 7.3 / 24.3 | 99.7 | 4.9 | 86.18 |
| 6 | Protocatechuic acid | 5.63 | 152.95 | 0.9991 | 0.5958 | 25.8 / 85.9 | 100.2 | 5.1 | 59.21 |
| 7 | Tannic acid | 6.46 | 182.95 | 0.9955 | 0.9075 | 10.2 / 34.2 | 97.8 | 5.1 | 161.35 |
| 8 | tr- caffeic acid | 7.37 | 178.95 | 0.9942 | 1.008 | 4.4 / 14.7 | 98.6 | 5.2 | 6.51 |
| 9 | Vanillin | 8.77 | 151.05 | 0.9995 | 0.4094 | 10.1 / 33.7 | 99.2 | 4.9 | 182.35 |
| 10 | p-Coumaric acid | 9.53 | 162.95 | 0.9909 | 1.1358 | 15.2 / 50.8 | 98.4 | 5.1 | 72.46 |
| 11 | Rosmarinic acid | 9.57 | 358.9 | 0.9992 | 0.522 | 10.4 / 34.8 | 101.7 | 4.9 | 0.00 |
| 12 | 4-OH Benzoic acid | 11.72 | 136.95 | 0.9925 | 1.4013 | 3.0 / 10.0 | 106.2 | 5.2 | 22.66 |
| 13 | Salicylic acid | 11.72 | 136.95 | 0.9904 | 0.6619 | 4 / 13.3 | 106.2 | 5 | 18.28 |
| 14 | Coumarin | 12.52 | 146.95 | 0.9924 | 0.4203 | 9.1 / 30.4 | 104.4 | 4.9 | 0.00 |
| 15 | Rutin | 10.18 | 609.1 | 0.9971 | 0.8146 | 17.0 / 56.6 | 102.2 | 5 | 41.70 |
| 16 | Hesperidin | 9.69 | 611.1 | 0.9973 | 0.1363 | 21.6 / 71.9 | 100.2 | 4.9 | 0.00 |
| 17 | Hyperoside | 10.43 | 463.1 | 0.9549 | 0.2135 | 12.4 / 41.4 | 98.5 | 4.9 | 0.00 |
| 18 | Myricetin | 11.94 | 317 | 0.9991 | 2.8247 | 9.9 / 32.9 | 106 | 5.9 | 2.35 |
| 19 | Fisetin | 12.61 | 284.95 | 0.9988 | 2.4262 | 10.7 / 35.6 | 96.9 | 5.5 | 0.00 |
| 20 | Quercetin | 14.48 | 300.9 | 0.9995 | 4.3149 | 2.0 / 6.8 | 98.9 | 7.1 | 0.00 |
| 21 | Naringenin | 14.66 | 270.95 | 0.9956 | 2.02 | 2.6 / 8.8 | 97 | 5.5 | 19.01 |
| 22 | Hesperetin | 15.29 | 300.95 | 0.9961 | 1.0164 | 3.3/ 11.0 | 102.4 | 5.3 | 0.23 |
| 23 | Luteolin | 15.43 | 284.95 | 0.9992 | 3.9487 | 5.8 / 19.4 | 105.4 | 6.9 | 0.17 |
| 24 | Kaempferol | 15.43 | 284.95 | 0.9917 | 0.5885 | 2.0 / 6.6 | 99.1 | 5.2 | 0.00 |
| 25 | Apigenin | 17.31 | 268.95 | 0.9954 | 0.6782 | 0.1 / 0.3 | 98.9 | 5.3 | 0.09 |
| 26 | Rhamnetin | 18.94 | 314.95 | 0.9994 | 2.5678 | 0.2 / 0.7 | 100.8 | 6.1 | 14.97 |
| 27 | Chrysin | 21.18 | 253 | 0.9965 | 1.553 | 0.05 / 0.17 | 102.2 | 5.3 | 0.00 |

**[a]RT: Retention time**
[b]Parent ion (m/z): Molecular ions of the standard compounds (mass to charge ratio)
[c]R2: coefficient of determination
[d]RSD: relative standard deviation
[e]LOD/LOQ (µg/L): Limit of detection/Limit of quantification
[f] U (%): Percent relative uncertainty at 95% confidence level (k=2).
[g] Values in µg/g (w/w) of plant methanol extract

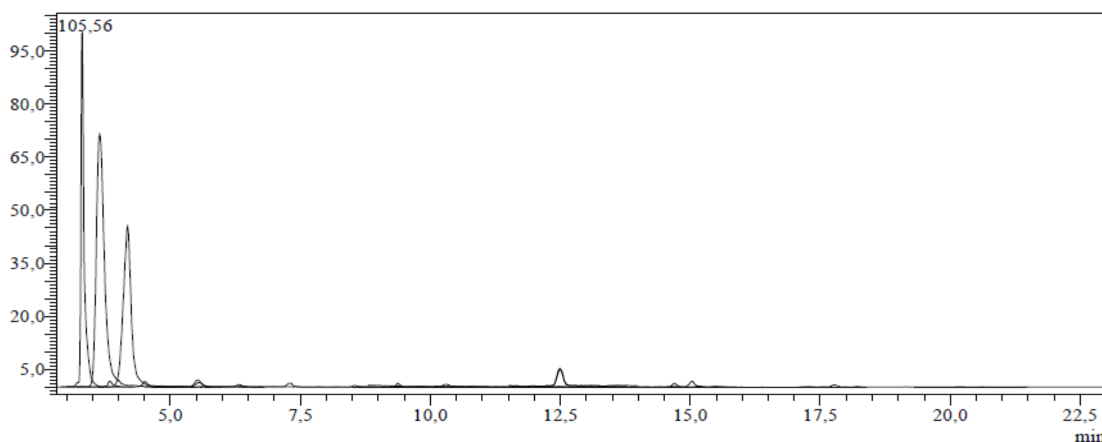


**Figure 2. Analytical parameters, identification and quantification of phenolic compounds of ethanol root extract of *Quercus infectoria* Oliv by LC ESI MS/MS.**

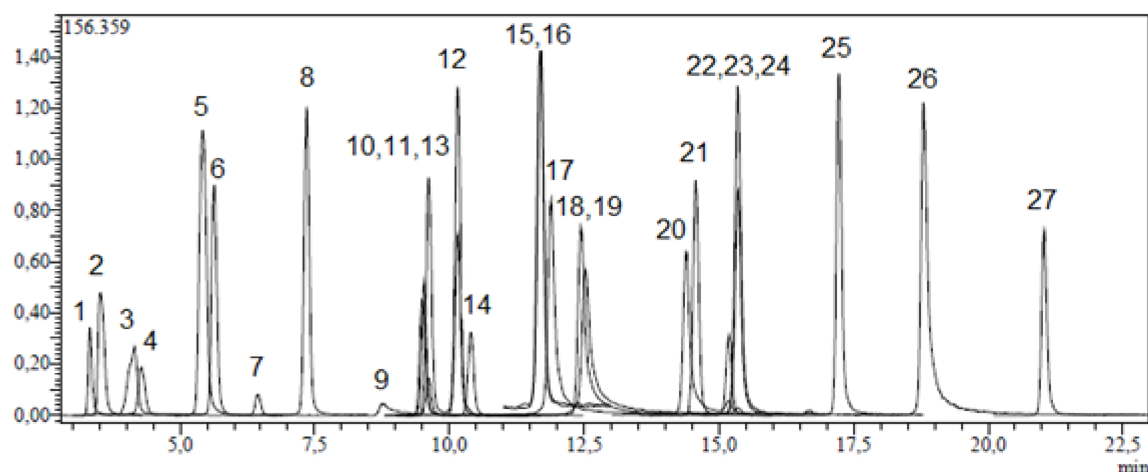


Figure 3. LC-MS/MS chromatogram for reference phytochemical compounds:

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.

## Discussion

In general, advanced yields were obtained by warm water extraction from all tissues. Actually, extraction yields with warm water are 2–3 times higher than those got by ethanol [27]. The effects of solvents polarity on extraction yield both qualitatively and quantitatively was confirmed by Franco et al.. This difference could be explained by a higher mass transfer due to the higher temperature of water extraction [28].

Many types of research had been studied and reported the importance of tannin and its variation. Their activity is possible because of their capability to connect with extracellular and soluble proteins or combine with the cell wall of fungi. The character of these compounds may disrupt fungal membranes [29].

Radical scavenging activity mechanism of root extracts could be related to the occurrence of polyphenolic compounds. It has previously been showed that the polyphenolic compounds are in charge of radical scavenging activity because of their hydrogen atom endowment to active free radicals [30].

The variance in amount levels of phenolic compounds could be due to differing methods of extraction [31]. Indeed, it could be due to the polyphenolic content of the roots being greatly affected by environmental factors as well as edaphic factors like soil type, sun exposure, rainfall, altitude and hightide, soil nutrients. etc. [32].

It has been conducted to elucidate the components responsible for the antimicrobial activity as well as any pharmacological or toxicological properties that such extracts might have [33].

## Conclusion

This research discovered that the extracts can possess' antioxidant activity and comparable to those got from commercial antioxidant. Indeed, in the present study indicates that the non-phenolic acid, phenolic acids and flavonoids are present in the root of *Quercus infectoria*. The occurrence of flavonoids in huge quantity is rationally proportional to the antioxidant activity, so it is evidently showed that occurrence of flavonoids will prove the antioxidant activity and promote a drug for treatment of various infectious disease. The strong antioxidant and antibacterial activities of *Q. infectoria* extracts are probably due to the presence of the bioactive compound in the plants.

The results showing big sources of quinic acid, gallic acid and rutin in root which might give an original source of this biological antifungal activity and antioxidant. The results from the current study recommended that root extract an ability source to grow as anti-candidiasis. It is intriguing to note that crude extracts of *Q. infectoria* root hold an antifungal potential, which might be further explored to be used as an alternative for treatment and control of some fungal infections. Further investigations are needed to develop a standardized *Q. infectoria* gall extract and to understand its mechanism of antifungal activity.

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