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In-vitro Assessment of Antifungal and Antioxidant Activities of Olive Leaves and Fruits at Various Extraction Conditions

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Abstract

Background and Objective: Nowadays, there is a growing interest for use of plant-based products such as extracts in various industrial sectors. Therefore, optimization of conditions for ideal extraction of bioactive compounds is highly important. Olive leaves and fruits include biophenols, which can be used as natural antimicrobial and antioxidant agents. Therefore, extraction of these bioactive compounds can create value-added products, which can be used as natural preservatives in food industries. The aim of this study was to investigate effects of various extraction parameters (type of solvent, solvent volume, temperature, time and pH) on *in-vitro* antioxidant and antifungal activities of Iranian olive leaf and fruit extracts against five *Candida* species.

Material and Methods: Olive fruit and leaf extracts were achieved using maceration method at various extraction conditions. Antioxidant activity of the prepared extracts was assessed by cupric reducing antioxidant capacity method. The phenolic profile in olive leaf extract was assessed using high performance liquid chromatography. Antifungal activity of the olive leaf extract was assessed using disk diffusion method and minimum inhibitory concentration and minimum fungicidal concentration values.

Results and Conclusion: Results showed that the highest antioxidant activity was recorded in olive leaf extract prepared by 100 ml of 96% ethanol at pH 7 and 80 °C for 6 h. Moreover, HPLC analysis of the ethanolic olive leaf extract showed that oleuropein was the major compound of the extract. Antioxidant activity of the olive leaf extract was higher than that of the fruit extract in various conditions. Regarding antifungal activity, the olive leaf extract showed a higher activity, compared to olive fruit extract at all concentrations. In olive leaf extract, the highest (62.5 µg ml^{-1}) and the lowest minimum fungicidal concentration (15.6 µg.ml^{-1}) values were reported for *Candida tropicalis* and *Candida albicans*, respectively. The minimum fungicidal concentration of the olive leaf extract was 250 µg ml^{-1} for *Candida albicans*, *Candida parapsilosis*, *Candida glabrata* and *Candida krusei* and 500 µg ml^{-1} for *Candida tropicalis*. It can be concluded that olive leaf extract is a source of antioxidant and antifungal substances with potential uses in food industries.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Plant extracts are complex mixtures from various parts of the plants, including fruits, leaves, flowers, woods, roots and seeds of plants with functional and medicinal characteristics [1]. Nowadays, the natural plant extract industry earns millions of euros worldwide [2]. Plant extracts exhibit various biological activities such as anti-inflammatory, antimicrobial and antioxidant activities due to the presence of bioactive compounds, majorly polyphenols [3]. Therefore, there is a growing interest to reuse these bioactive phytochemicals in various industrial sectors such as food, pharmaceutical and cosmetic sectors [4]. Olive (*Olea europaea* L.) is an evergreen tree of the Oleaceae family and a subset of flowering plants (Angiospermae). This plant is native to temperate tropics and one of the oldest known cultivated

plants [5]. Olive trees are principally detected in coastal areas of the eastern Mediterranean basin, the contiguous coastal areas of Southern Europe, Northern Iran (at the south end of the Caspian Sea), Western Asia and North Africa [6]. The most important part of the olive trees includes its fruit that consists of water (~50%), oil (~22%), proteins (1.6%), carbohydrates (19.1%), cellulose (5.8%), inorganic substances (1.5%) and phenolic compounds (1-3%). There are 4 major biophenols in olive fruits, including (i) phenolic acids and alcohols, (ii) flavonoids, (iii) lignans, and (iv) secoiridoids. Secoiridoids e.g. oleuropein, ligstro-side and simple phenolics such as tyrosol and hydroxytyrosol have been reported as the major phenolics of olive fruits [7,8].

Olive leaves are the byproducts achieved after harvesting the olive fruits, constituting 10% of the entire olive harvest weight and 25 kg/tree during olive tree pruning [9]. Olive leaves contain valuable bioactive compounds with antioxidant, antimicrobial, anti-inflammatory, antihypertensive, antithrombotic and hypoglycemic characteristics [10]. Oleuropein and hydroxytyrosol are the most abundant phenolic compounds in olive leaves. Oleuropein is one of the secoiridoids and an essential biophenolic compound. It is a heterozygous ester of beta-glucose and 3,4-dihydroxyphenyl ethanol or hydroxy tyrosol. Hydrolysis of oleuropein can produce elenolic acid, hydroxytyrosol, tyrosol and glucose. Oleuropein is majorly in the form of aglycone, which makes its sugar part insoluble in oil [11]. Antioxidant and antimicrobial activities of the bioactive compounds in olive fruits and leaves have been reported in several studies. Almatroodi et al. [12] investigated the antioxidant activity of ethanolic olive fruit pulp extract and reported the highest DPPH free radical scavenging activity at the level of 600 µg ml^{-1}. Protective effects of the phenolic extract of table olive on Caco-2 cells against oxidative stress and membrane damage were induced by tert-butyl hydroperoxyde [13]. In another study, antioxidant and antimicrobial activities of olive leaf extracts by ultrasound-assisted extraction were assessed and high antioxidant and antibacterial activities against *Yersinia enterocolitica* and *Staphylococcus aureus* were observed [14]. Other studies have reported antimicrobial activity of olive leaf extract against *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Salmonella enteritidis, Salmonella typhimurium*, *Klebsiella pneumonia Bacillus* cereus, *B. subtilis*, *Candida albicans*, *Pseudomonas fluorescens* and *Corynebacterium* Sp. [15-17].

These antioxidant and antimicrobial activities of olive fruit and leaf extracts make them excellent sources of bioactive ingredients for use in food industries. So, extraction of compounds from these materials and their uses in food systems can enhance sustainability, decrease waste disposal and create value-added products [18]. Extraction is an essential determinative step for the use of cellular bioactive compounds, which should perfectly be designed to increase yield of the bioactive compounds in the final products. The processing parameters, including sample preparation, extraction time, temperature, solvent type and solvent ratio can affect level of extractability and functionality of the final extract [19,20]. Hence, the aim of this study was to investigate effects of various extraction conditions, including solvent type, solvent volume, temper-ature, pH, and extraction time on antioxidant activity of Iranian olive leaf and fruit extracts. Moreover, antifungal effects of olive fruit and leaf extracts were assessed against five species of *Candida*, including *C. albicans, C. glabrata, C. parapsilosis, C. tropicalis* and *C. krusei*.

2. Materials and Methods

2.1. Materials

Neocuproine powder, ammonium acetate powder, cupric sulfate pantahydrate, methanol, acetonitrile, propyl gallate, trifluoroacetic acid, high-performance liquid chromatography (HPLC) grade acetonitrile, Mueller-Hinton agar, methylene blue powder, glucose powder, Roswell Park Memorial Institute (RPMI) media and NaOH powder were purchased from Merck, Darmstadt, Germany. Ultrapure water was produced using Direct-Q 3 UV with a pump system (Millipore, Molshein, France). Oleuropein standard and 3-(n-morpholino) propanesulfonic acid (MOPS) buffer powder were purchased from Sigma, St. Louis, MO, USA. Hydrochloric acid was provided by Loba Chemie, India, and 96% ethanol from Hamunteb, Iran.

2.2. Sample collection

Fresh green olive (*Olea europaea*) fruit and leaf samples were collected from trees in Qom Province, Iran, October 2023. A voucher specimen of each cultivar was deposited in the Herbarium of Tehran University of Medical Sciences, Tehran, Iran, with the number of 1036.

2.3. Extraction procedure

The olive fruits and leaves were chopped into small pieces and stored at 4 °C until further use. Extracts were achieved via maceration of 5 g of olive leaves and 5 g of olive fruits at various extraction conditions using Erlenmeyer flasks. Flasks were covered with aluminum foil to avoid light exposure and solvent evaporation. Extracts were filtered using Whatman filters no. 1 (Whatman, UK) and then stored at 4 °C. Solvents used for the extraction included 96% ethanol, 80% ethanol, 50% acetonitrile, tap water and distilled water (DW). Extraction was carried out using volumes of 10, 35, 50, 75 and 100 ml. Extraction temperatures included ambient (~25), 40, 60 and 80 °C. The investigated range of pH included 1, 3, 5, 7, 9 and 11, which were adjusted with hydrochloric acid (0.1 N) or sodium hydroxide (0.1 N) solution. Duration of extraction was assessed within 1, 2, 3, 4, 5 and 6 h [21].

2.4. Assessment of antioxidant activity

The CUPric reducing antioxidant capacity (CUPRAC) method by Hidayat et al. [22] was used to assess antioxidant activity of each extract. Technical abbreviations were expl-

ained upon first use. In the CUPRAC assay, samples were mixed with $CuSO_4$ and neocuproine, resulting in the reduction of Cu (II) to Cu (I) through the action of electron-donating antioxidants. Then, absorbance was recorded at 450 nm after a 30-min incubation time. Results were expressed as milligrams of propyl gallate per liter of sample.

2.5. High-performance liquid chromatography analysis of extract

Briefly, HPLC analysis was carried out for the analytical qualification and quantification of phenolic compounds in ethanolic olive leaf extract. Extract was filtered through 0.45-mm membrane filters and injected to the HPLC system, including a Knauer HPLC (Knauer, Berlin, Germany) equipped with a Smartline pump 100, a Smartline photo-diode array detector 2800, a Smartline autosampler 3950 and a reverse phase µBondpack C18 column (250 × 4.6 mm I.D.) (Waters, Milford, MA, USA). Mobile phase consisted of two parts of buffer A (water and trifluoroacetic acid, 0.50%) and buffer B (acetonitrile and trifluoroacetic acid, 0.50%). The mobile phase flow rate was 1 ml min^{-1} and the injection volume of each sample was 20 µl. Data were collected and processed using EZ-chrom data analysis software. All phenolic compounds were identified by comparing their retention times with those of standards (hydroxytyrosol, apigenin-7-O-glucoside, oleuropein and oleuroside) [19].

2.6. Assessment of antifungal activity

Microorganisms were provided by the Microorganism Bank of the Department of Mycology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. This included five species of *Candida* (*C. albicans*, GenBank MW737-375.1, *C. glabrata*, GenBank MW741897.1; *C. krusei*, Gen Bank MW714851.1; *C. tropicalis*, GenBank MW737374.1 and *C. parapsilosis*, GenBank MW828315.1). The *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were used as reference strains. To prepare the fungal suspension under sterile conditions, a loop was used to remove a fungal colony (equivalent to five micro clones) from a fresh culture. Colonies were transferred to sterile tubes containing 5 ml of sterile saline and mixed well by vortex. Then, cell count was carried out using spectrophotometry at 630 nm and Neubauer slide. Fungal suspension was prepared at a concentration of 10^6 (colony forming unit or CFU ml^{-1}). Antifungal activity of the extracts was assessed using standard methods by Clinical and Laboratory Standards Institute of disk diffusion (M44-A) and microdilution broth (M27-A3) methods [23].

2.6.1. Disk diffusion method

Sterile swabs were soaked in a fungal suspension. The swaps were evenly and linearly streaked in all directions onto Mueller-Hinton agar. After 10 min when the fungal suspension was absorbed, discs were transferred on the media. Three raw discs for each olive leaf and fruit extracts with doses of 10, 20, and 30 µl were used. For the control disc, 20 µl of 96% ethanol were used. Plates were incubated for 35–48 h at 35 °C. Then, diameter of the growth inhibition zones was measured in mm [24].

2.6.2. Broth microdilution method (assessment of minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC)

Anticandidal activity of the extracts was assessed through a broth microdilution method of two-fold serial dilutions in RPMI 1640 media as described in M27-A3 reference method of Clinical and Laboratory Standards Institute [25]. The primitive fungal suspension was initially diluted at a ratio of 1:100 in sterile physiological saline and vortexed. Then, solution was further diluted at a ratio of 1:20 in RPMI media and vortexed. The final fungal suspension concentration ranged from 0.5 to 2.5 $\times 10^3$. For each extract and each Candida spp., two replications were set on a 96-well plate. Column 11 was used as positive control (without extract), while Column 12 was used as negative control (without yeast). First, 100 µl of RPMI media were added to Columns 2 to 11. In Column 12, 200 µl of RPMI media were added. In Column 1, 200 µl of the extract were added, followed by the serial dilution operation. To achieve a 1/2 fraction of the extract, extract content was removed from the first well of Column 1 into the second well of Column 2. This procedure was repeated up to Column 10; then, 100 µl were poured from Column 10 (resulting in a fraction of 1/512 of the primary extract). Moreover, 100 µl of the prepared fungal suspension were added to Columns 1 to 11. Plates were incubated at 35-37 °C for 24-48 h. To assess the MFC, 100 µl of the sample were collected from the wells with MIC concentration as well as from three wells with concentrations higher than MIC and recultured in sub-dextrose agar media. After incubation at 30 °C for 24 h, the colony growth rate and cell count were compared with those of control and blank samples (Column 12) and the minimum concentration that inhibited the yeast growth was reported as the MFC. The MIC of the test compound for each isolate was assessed as the lowest concentration, resulting in significant decreases in the fungal growth (inhibition ≥50%), compared to the growth control. The MFC was recorded as≥99.9% reductions in the number of CFUs from the starting inoculum count, while fungistatic activity was reported as≤99.9% reductions.

2.7. Statistical analysis

All experiments were carried out in triplicate and data were present as mean ±SD (standard deviation). Statistical analysis of data was carried out using one-way analysis of variance (ANOVA) and Duncan's multiple range test to compare the means and SPSS statistical software v.22 (SPSS, Chicago, IL, USA). Differences at $p<0.05$ were recorded as significant [26].

3. Results and Discussion

3.1. Effects of the extraction conditions on antioxidant activity of the olive leaf and fruit extracts

Relationships between polyphenols and antioxidant activity have been reported in several studies [27-29]. Naturally, dietary polyphenols act as efficient free radicals and reactive oxygen species scavengers due to the presence of aromatic ring, several hydroxyl groups and highly conjugated systems [30]. These are capable of neutralizing reactive oxygen species or quenching cellular oxidative stress, allowing them to prevent oxidative damages of biomolecules (e.g. lipids, proteins and DNA) and thereby diminish tissue inflammation [31]. Effects of type of solvents on antioxidant activity of the olive fruit and leaf extracts are illustrated in Figure 1. The highest antioxidant activity was linked to extracts; in which, 96% ethanol was used as solvent. Then, 80% ethanol, 50% acetonitrile, DW and tap water extracts showed higher antioxidant activities, respectively (Figure 1). It has been reported that antioxidant activity of the plant extracts is associated with the type of solvents used for the extraction. Polar solvents are frequently used for the recovery of polyphenols that are correlated with high antioxidant capacities. Ethanol, acetone, ethyl acetate, methanol and aqueous mixtures of these solvents have extensively been used for the extraction of compounds from plants with antioxidant characteristics [32]. Borges et al. [32] used various techniques and solvents for the preparation of olive extract and reported the highest antioxidant activity in extracts achieved by ethanol and acetone. In a study by Cho et al. [9], DPPH radical scavenging activity of 33.84, 32.97, 33.27 and 31% were observed for 90 vol% methanol, 50 vol% methanol, 70 vol% methanol, aqueous ethanol and aqueous acetone.

Moudache et al. [33] prepared olive leaf and cake extracts with various solvents [ethanol and acetone (both 50 and 70%) as well as DW] and detected that the solvent polarity affected total phenolic content and then antioxidant activity. The lowest DPPH radical scavenging activity was achieved with DW that was attributed to the absence of oleuropein in leave and cake extracts and absence of luteolin-7-O-glucoside in cake aqueous extract, respectively. As shown in Figure 1, leaf extract included a higher antioxidant activity than that the fruit extract did using various solvents.

Based on Figure 2, increasing the solvent volume enhanced the antioxidant activity. It has been stated that high solvent volumes can intensify substance transfer and assist solubility by increasing contact surfaces of the plant materials with the solvent, improving the extraction efficiency [34]. With increasing the extraction temperature, increases in the antioxidant activity were observed (Figure 3).Under similar extraction conditions, the leaf extract included more antioxidant activity than that the fruit extract did. Similar results have been reported by Quiles-Carrillo et al. [35] for carob pod extracts. Higher extraction temperatures improved DPPH inhibition from 68 to 89%. High temperatures accelerated intermolecular interactions and promoted molecular motion, which increased solubility of the bioactive compounds [34]. At higher temperatures, solubility and diffusion coefficients are higher while solvent viscosity and surface tension are lower with weaker phenolic-protein and phenolic-polysaccharide linkages that facilitate migration of phenolic compounds into the solvent [36]. Based on Figure 4, by increasing the extraction time, antioxidant activity increased in the two extracts. Similar results have been reported by Piechowiak et al. [37] for increased antioxidant activities of yellow onion peals by extending the extraction time. It has been stated that increasing contact time of the solvent with plant materials might improve diffusion of the antioxidant compounds [38]. According to Figure 5, pH 7 was the optimum pH for obtaining the highest antioxidant activity in olive leaf and fruit extracts.

3.2. Identification of phenolic compounds in olive leaf extract by high-performance liquid chromatography

Olive leaves contain various phenolic compounds, including simple phenols, flavonoids and secoiridoids [18]. Phenolic profile in olive fruits and leaves is strongly affected by the cultivar, region, climate, irrigation, and time of harvest, ripeness as well as post-harvest processing [5]. The HPLC analysis of olive leaf extract is shown in Figure 6.

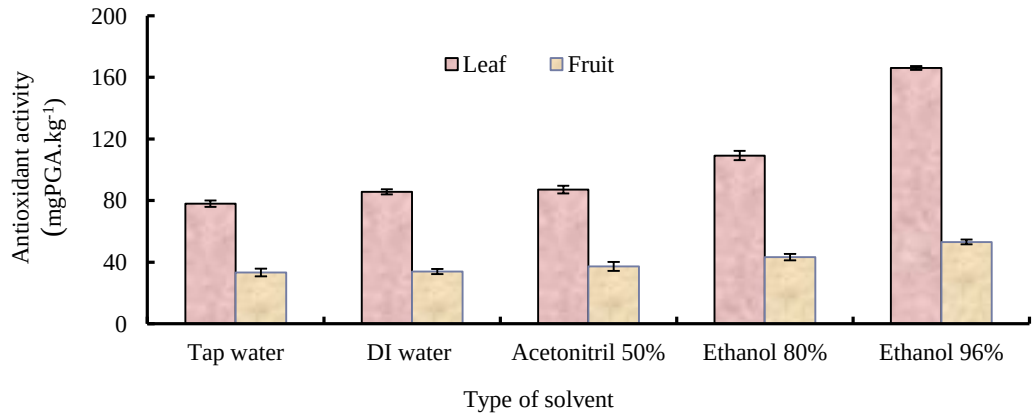

Figure 1. Effects of type of solvents on the antioxidant activity of olive leaf and fruit extracts with extraction conditions of sample weight, 5 g; temperature, 25 ºC, pH 1; volume of solvent, 50 ml; and extraction time, 1 h.

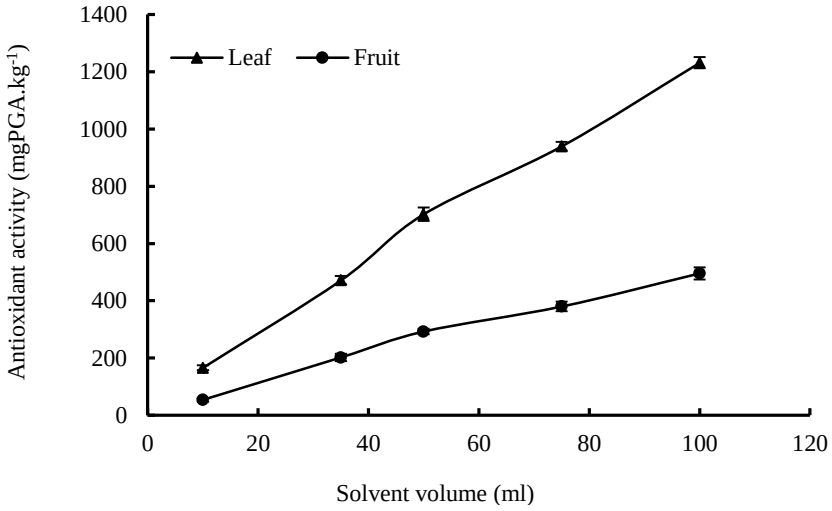

Figure 2. Effects of the solvent volume on the antioxidant activity of olive leaf and fruit extracts with extraction conditions of sample weight, 5 g; temperature, 25 ºC; pH 1; solvent, ethanol 96%; and extraction time, 1 h.

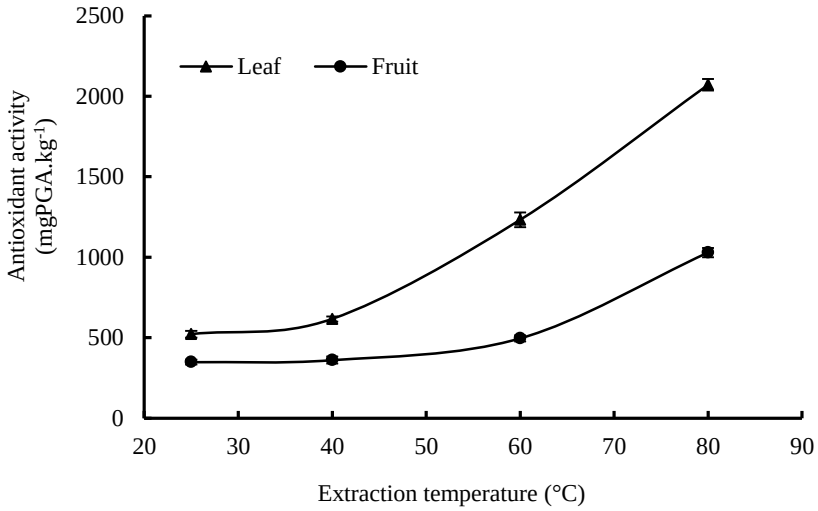

Figure 3. Effects of temperature on the antioxidant activity of olive leaf and fruit extracts with extraction conditions of sample weight, 5 g; solvent volume, 50 ml; pH 1; solvent, ethanol 96%; and extraction time, 1 h.

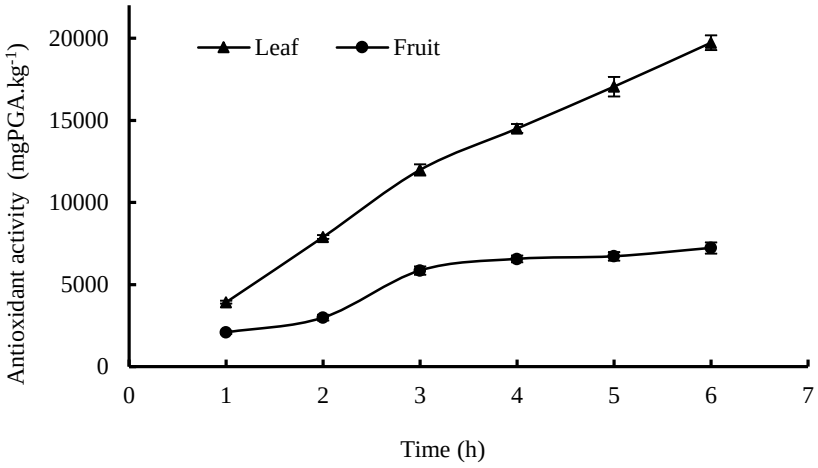

Figure 4. Effects of extraction time on the antioxidant activity of olive leaf and fruit extracts with extraction conditions of sample weight, 5 g; pH 7; temperature, 80 ºC; volume of solvent, 50 ml; and solvent, ethanol 96%.

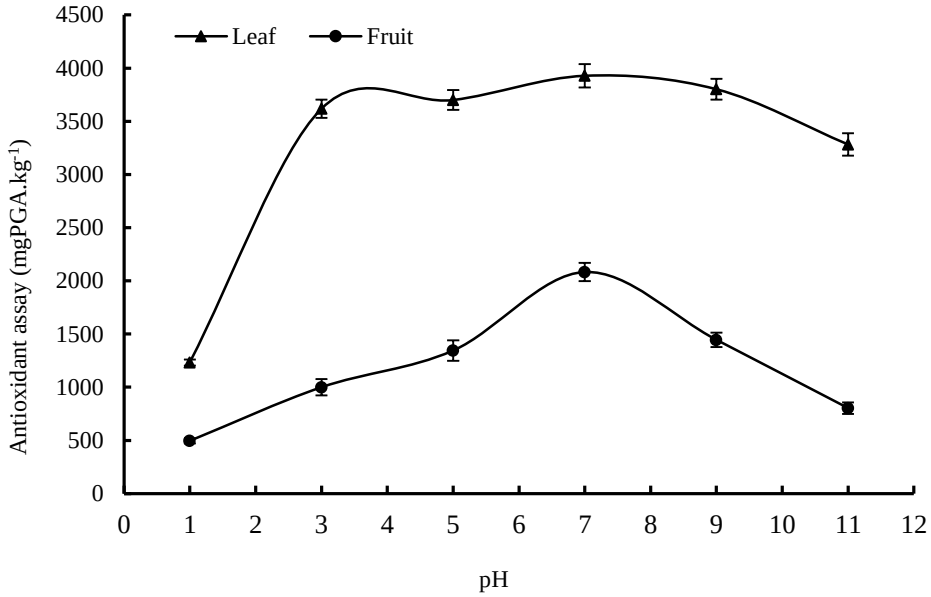

Figure 5. Effects of pH on the antioxidant activity of olive leaf and fruit extracts with extraction conditions of sample weight, 5 g; temperature, 80 ºC; solvent volume, 50 ml; solvent, ethanol 96%; and extraction time, 1 h.

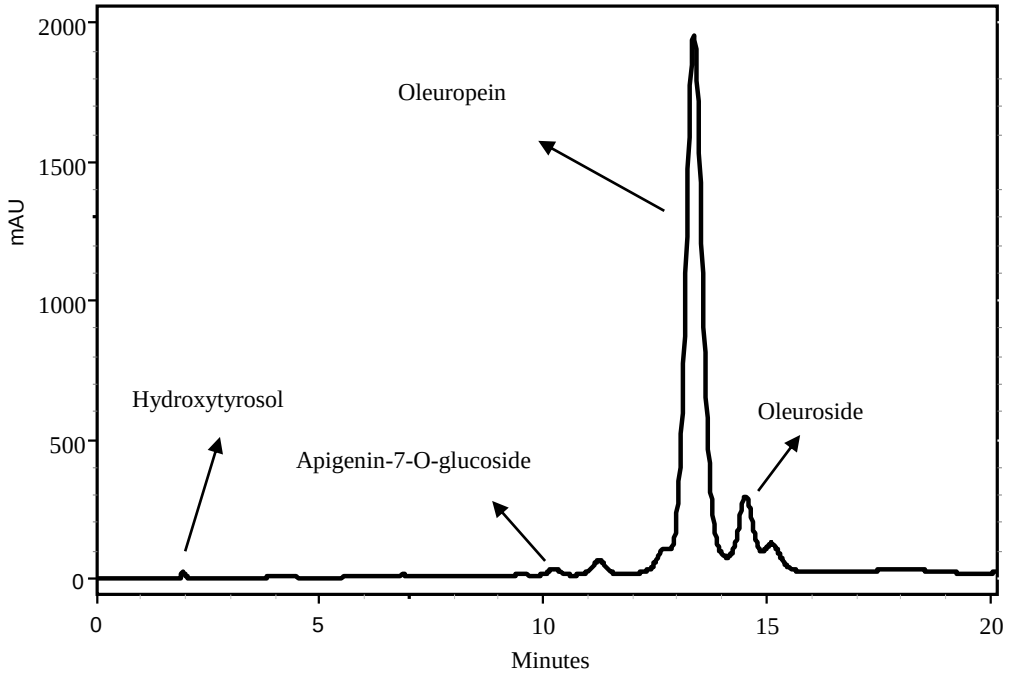

Figure 6. Chromatogram of the phenolic compounds in the optimum olive leaf extract analyzed using HPLC.

Four major peaks were identified, including hydroxytyrosol, apigenin-7-O-glucoside, oleuropein and oleuroside. Final concentration of the phenolic compounds in olive leaf and fruit extracts included 4000 and 1500 mg l^{-1}, respectively. As shown in Figure 6, oleuropein was the major compound in the extract. The highlighted polyphenols have previously been reported in several studies. Sanchez-Gutierrez et al. [39] identified hydroxytyrosol, 4 flavonoids (luteolin, luteolin-7-O-glucoside, apigenin and apigenin-7-O-glucoside), oleuropein and verbascoside in olive leaf extracts using Soxhlet and microwave-assisted extractions. It was stated that the types of method and solvent and interactions of the two factors included significant effects on the total phenols. In a study by Medina et al. [40], oleuropein was the most abundant compound (more than 88-94% of the total phenolic compounds) identified in olive leaf extracts.

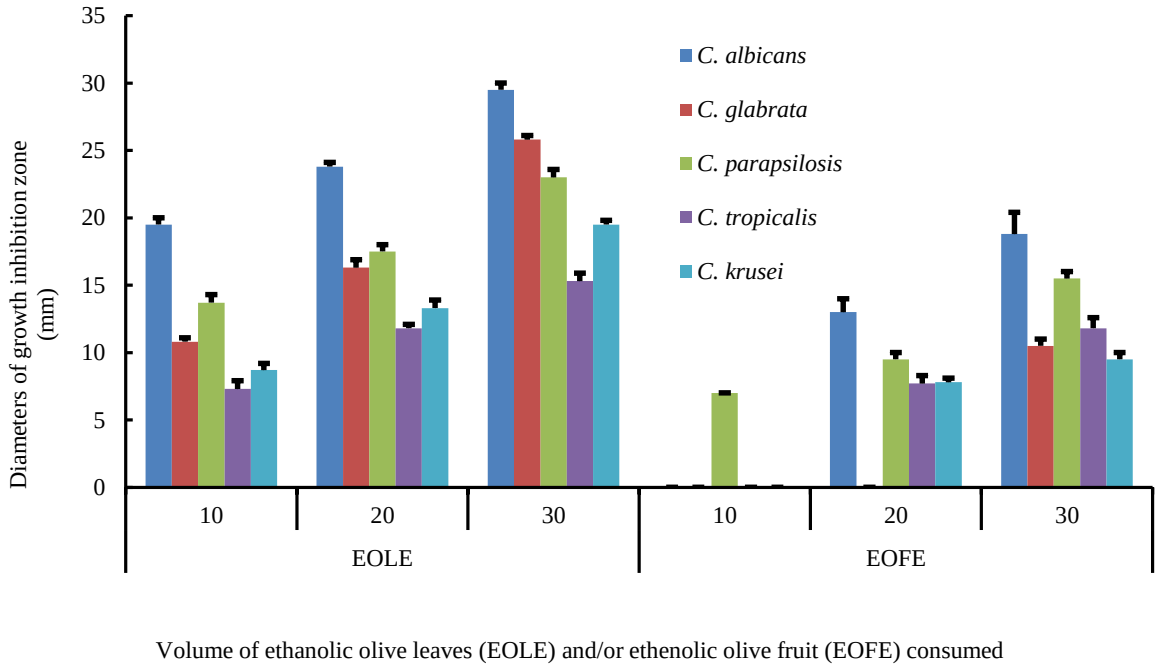

Figure 7. Antifungal activities of the ethanolic olive leaf and fruit extracts using disc diffusion assay.

Ligustroside, hydroxytyrosol 1-glucoside and hydro-xyl-tyrosol 4-glucoside were present at levels of 2-7, 2-1 and 0.5-2.5% of the total phenolic compounds, respectively. Similarly, characterization of hydroethanolic leaf extracts from six Mediterranean olive cultivars showed 66 individual phenolic compounds majorly, including oleuropein, hydroxytyrosol, oleoside/secologanoside, verbascoside, rutin, luteolin and ligstroside as well as their derivate [41]. In the study of Ghomari et al. [19], oleuropein (29.5 mg.g^{-1}□±0.10□) was the major compound in 80% ethanolic olive leaf extracts followed by coumaric acid and gallic acid. Rutin, luteolin, hydroxytyrosol, tyrosol and quercetin were reported as the minor phenolic compounds.

3.3. Antimicrobial activity of the olive leaf and fruit extracts

Results showed that the olive leaf extract included a higher antimicrobial activity, compared to that the olive fruit extract did at all concentrations (Figure 7). At volumes of 10 and 20 µl of olive leaf extract, *C. albicans, C. para-psilosis, C. glabrata, C. krusei*, and *C. tropicalis* were the most susceptible species. In general, 10 µl of the olive fruit extract inhibited growth of *C. parapsilosis*. At a volume of 20 µl of the olive fruit extract, no inhibition activity was observed against *C. glabrata*.

Table 1 presents MIC and MFC values of the ethanolic olive leaf and fruit extracts. In the olive leaf extract, the highest (62.5 µg ml^{-1}) and the lowest (15.6 µg ml^{-1}) MIC values were reported for *C. tropicalis* and *C. albicans*, respectively. The MFC of olive leaf extract was 250 µg ml^{-1} for *C. albicans, C. parapsilosis, C. glabrata* and *C. krusei* and 500 µg ml^{-1} for *C. tropicalis*. In the olive fruit extract, MIC values of 500 µg ml^{-1} for *C. albicans* and *C. parapsilosis* and 1000 µg ml^{-1} for *C. glabrata, C. krusei* and *C. tropicalis* were reported. The MFC values of 2000 µg ml^{-1} for *C. albicans* and *C. parapsilosis* and 4000 µg ml^{-1} for *C. glabrata, C. krusei* and *C. tropicalis* were reported as well. It has been reported that the antimicrobial potential of olive is primarily attributed to the presence of secoiridoid compounds and their derivatives, including secoiridoids oleuropein, demethyloleuropein and ligstroside [5].

Table 1. Minimal inhibitory concentration and minimal fungicidal concentration of the olive leaf and fruit extracts

Candida Species	EOLE* (µg ml^{-1})		EOFE (µg ml^{-1})	
	MIC	MFC	MIC	MFC
C. albicans	15.6 ± 0.26	250 ± 1.24	500 ± 2.16	2000 ± 1.63
C. glabrata	31.2 ± 1.03	250 ± 1.21	1000 ± 2.05	4000 ± 2.44
C. parapsilosis	31.2 ± 0.11	250 ± 1.25	500 ± 1.63	2000 ± 2.16
C. tropicalis	62.5 ± 0.73	500 ± 2.94	1000 ± 2.86	4000 ± 1.24
C. krusei	31.2 ± 0.69	250 ± 1.20	1000 ± 2.62	4000 ± 2.49

* EOLE: ethanolic olive leaf extract, EOFE: ethanolic olive fruit extract
MIC: minimum inhibitory concentration; MFC: minimum fungicidal concentration

Khan et al. [42] studied antifungal activity of three various leaf extracts of a wild variety of *Olea europaea* using well diffusion method. It was observed that the aqueous and ethanolic extracts included higher antifungal activities against *Aspergillus flavus* than that the ethyl acetate extract did. For *A. niger*, ethanolic and ethyl acetate extracts were more efficient than the aqueous extract. In another study, antifungal activity of oleuropein against *C. albicans* was investigated and results showed a MIC value of 12.5 µg ml^{-1}. It was revealed that oleuropein targeted virulence factors essential for the establishment of the fungal infection. It inhibited morphological conversion of *C. albicans* cells to their filamentous form. Moreover, oleuropein prevented activity of the secreted aspartyl proteinases from *C. albicans*, which were associated with its pathogenicity [43]. In a study by Drais et al. [44], hydroxytyrosol-enriched extracts from olive oil waste water inhibited growth and conidia germination of *Verticillium dahlia* during 24 h of incubation. This was attributed to the damages of cell functions and membrane integrity that resulted in loss of cytoplasmic constituents. It has been declared that oleuropein and ligstroside are antimicrobial precursors for smaller antimicrobial compounds, including aglycones, hydroxytyrosol, tyrosol and elenolic acid [5]. According to Ansari et al. [45], antimicrobial activity of the phenolic compounds is due to the synergistic effects of antioxidant and chelating effects of the hydroxyl groups in the structure that formed hydrogen bonds with cell proteins of the microorganisms. Therefore, it can be concluded that increased extraction yields of polyphenols from olive fruits and leaves with ethanol as well as naturally occurring combinations of these compounds with synergistic effects are the possible inhibitory mechanisms of action against the studied *Candida* species.

4. Conclusion

Olive leaves and fruits are the products with great potentials for creating value-added products due to the presence of phenolic compounds. In this study, olive fruit and leaf extracts were prepared at various extraction conditions, demonstrating that 96% ethanol was the most efficient solvent for the extraction of bioactive compounds with the highest antioxidant activity. Regarding other factors, the highest solvent volume (100 ml) and temperature (80 °C) at pH 7 were the best conditions for achieving the highest antioxidant activity. It was observed that olive leaf extract included a higher antioxidant activity than that the olive fruit extract did possibly due to the differences in contents of polyphenolic compounds in these two parts. Furthermore, HPLC analysis of the ethanolic olive leaf extract showed four major compounds, including hydroxytyrosol, apigenin-7-O-glucoside, oleuropein and oleuroside. Regarding antifungal activity, *C. albicans* and *C. tropicalis* were the most susceptible and the most resistant species. The olive leaf extract can be addressed as an inexpensive natural antioxidant and antimicrobial agent with potential uses in food industries. However, further studies should be carried out to investigate effects of other extraction methods on the functional characteristics of olive extracts as well as assessment of the antioxidant and antimicrobial activities in food products. Moreover, further studies should be carried out to interpret relationships between the structure and antioxidant and antimicrobial activities of the bioactive compounds in olive extracts.

5. Acknowledgements

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6. Conflict of Interest

Conceptualization, J.H and K.G.; methodology, A.S., J.H. and G.S.; investigation, S.M. and A.A; data curation, X.X.; writing-original draft preparation, S.M., H.H., A.A. and A.S.; writing-review and editing, J.H and K.G.; supervision, J.H and K.G.

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بررسی برون تن فعالیت‌های ضدقارچی و ضداکسایشی میوه و برگ‌های زیتون در شرایط گوناگون استخراج

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چکیده

سابقه و هدف: امروزه تمایل برای استفاده از فرآورده‌های گیاهی مانند عصاره‌ها در بخش‌های گوناگون صنعت رو به افزایش است. بنابراین، بهینه‌سازی شرایط استخراج مطلوب ترکیبات زیست‌فعال از اهمیت بالایی برخوردار است. برگ‌ها و میوه‌های زیتون حاوی بیوفنول‌هایی هستند که به‌عنوان عوامل ضدمیکروبی و ضداکسایشی طبیعی قابل استفاده هستند. بنابراین استخراج این ترکیبات زیست فعال می‌تواند محصولاتی با ارزش افزوده ایجاد کند که به‌عنوان نگهدارنده طبیعی در صنایع غذایی استفاده می‌شود. هدف از این مطالعه بررسی اثرات پارامترهای گوناگون استخراج (نوع حلال، حجم حلال، دما، زمان و pH) بر فعالیت ضداکسایشی و ضد قارچی در *شرایط برون تنی* عصاره‌های برگ و میوه زیتون ایران بر روی پنج گونه *کاندیدا* بود.

مواد و روش ها: عصاره‌های میوه و برگ زیتون به روش خیساندن در شرایط گوناگون استخراج تهیه شد. فعالیت ضداکسایش عصاره‌های تهیه شده با روش ظرفیت ضداکسایشی احیای مس ارزیابی شد. پروفایل فنلی عصاره برگ زیتون با استفاده از کروماتوگرافی مایع با کارایی بالا ارزیابی شد. فعالیت ضد قارچی عصاره برگ زیتون با استفاده از روش دیسک دیفیوژن و حداقل غلظت بازدارندگی و حداقل غلظت کشندگی قارچ مورد ارزیابی قرار گرفت.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که بیشترین فعالیت ضداکسایشی مربوط به عصاره برگ زیتون تهیه شده با ۱۰۰ میلی لیتر اتانول ۹۶ درصد در pH=۷ و ۸۰ درجه سانتی‌گراد به‌مدت ۶ ساعت، بود. علاوه براین، آنالیز عصاره اتانولی برگ زیتون با HPLC نشان داد که اولئوروپئین ترکیب اصلی عصاره است. فعالیت ضداکسایشی عصاره برگ زیتون در شرایط گوناگون بیشتر از عصاره میوه بود. از نظر فعالیت ضد قارچی، عصاره برگ زیتون در تمام غلظت‌ها فعالیت بیشتری نسبت به عصاره میوه زیتون داشت. در عصاره برگ زیتون، بیشترین ($۶۲/۵\mu g\ ml^{-1}$) و کمترین غلظت قارچ‌کشندگی ($۱۵/۶\ \mu g\ ml^{-1}$) به ترتیب برای *کاندیدا تروپیکالیس* و *کاندیدا آلبیکانس* گزارش شد. حداقل غلظت قارچ‌کشندگی عصاره برگ $۲۵۰\ \mu g\ ml^{-1}$ برای *کاندیدا آلبیکانس*، *کاندیدا پاراپسیلوزیس*، *کاندیدا گلابراتا* و *کاندیدا کروسی* و $۵۰۰\mu g\ ml^{-1}$ برای *کاندیدا تروپیکالیس* بود. از نتایج این تحقیق می‌توان نتیجه گرفت که عصاره برگ زیتون منبعی از ترکیبات ضداکسایشی و ضدقارچی با کاربردهای بالقوه در صنایع غذایی می‌باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- فعالیت ضدقارچی
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- استخراج
- اولئوروپئین
- زیتون

***نویسنده مسئول**
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