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Evaluation of Phytochemical, Antimicrobial, and Antioxidant Properties of Wild versus Cultivated Olive Leaves

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ABSTRACT

The relation between *Olea europaea* subspecies cuspidata and subspecies europaea in Saudi Arabia were investigated for determination of the bioactive compounds in olive leaves. The antioxidant and total phenolic content were demonstrated by HPLC/MS. Also, the antimicrobial agar gel well diffusion activity was done for ethanolic extracts. The study identified a great number of phenolic compounds out of which some anticancer compounds were identified. The content of olive leaves extract differs according to habitat (either wild or cultivated). The ethanolic extract of both types has high phenolic content (21.3 to 22.6 mg GA/gdw) and antioxidant activity (71% to 57%) for wild and cultivated leaf extracts, respectively. The phenolic profiles revealed the presence of plenty of flavonoids and phenolic compounds. The major polyphenol in the extracts is naringenin, which appears at peaks 8 with concentrations of 21.93 and 17.35 µg/mL for cultivated and wild leave respectively, which retention times 14.99 and 14.98. The antimicrobial activity showed that the leaves extract have strong antimicrobial activity against *Bacillus subtilis*, with the highest inhibition zones (28 and 26 mm) for wild and cultivated leaf extracts, respectively. This is considered as valuable data about the potential for industrial mass production of polyphenol.

1. INTRODUCTION

Olive (*Olea europaea* L.) is one of the most important crops in Mediterranean nations. The olives are utilized for table utilization as well as a significant wellspring of oil. The olive tree has a long practice of memorable and social importance (**Figure 1**). *Olea europaea* trees spread in the Mediterranean Basin where they are native as well as in other locales with a Mediterranean environment where they have been presented [1]. All developed and wild olive trees have a place with two organic assortments: The wild olive trees or oleaster relate to *Olea europaea* subsp. cuspidata. The developed olive trees relate to *Olea europaea*

subsp. europaea [2] and include olive cultivars developed for oil or table olives. The most widely recognized and famously utilized is the items acquired from olive tree (fruit, oil, leaves) [3].

Olive leaves have also been combined along with over-matured olives to produce oils with a more pronounced flavor and better oxidation resistance [4], utilized straightforwardly as a complement to olive oil [5], and their polyphenol content was employed dietetic capsules as well as food supplements formulation [6]. Olive leaves accumulate the interest of established researchers and businesses worldwide as their wellbeing advancing advantages are continually being shown by a steadily expanding number of logical information [7]. They are considered as results of olive cultivating, and one of the main exercises in the Mediterranean area, addressing practically 10% of the absolute weight of materials showing up to the olive factory. Historically, olive leaf was used for the treatment of malaria and associated fever [8]. Nevertheless, just few investigations have focused on the accumulation of polyphenol constituents within olive leaves, for example, hydroxytyrosol, quercetin-3-rutinoside, acteoside, cynaroside, oleuropein and its derivatives (ligstroside and oleuropein aglycone) [9], and different mixtures, for example, quinic corrosive [10]. These multitudes of parts have been shown to be advantageous in human wellbeing on account of their cancer prevention agent legitimacies. Olive leaf extracts have been shown to exhibit antihypertensive [11], antitumor [12], hypoglycemic, and antibacterial effects against *Helicobacter pylori* and *Campylobacter jejuni* [13], as well as hypocholesterolemic effects [14]. This vast number of positive implications appears to be linked to the potent antioxidant properties of the lower molecular weight polyphenols, like oleuropein, and polar molecules, like quinic acid, being the most common [15, 16]. As of late, a few investigations zeroed in on substance of the olive leaves and extraction of these high-added esteem compounds from olive leaves. Dissolvable extraction is a positive interaction since heat-touchy materials can be recuperated at low temperatures. Thus, it is liked for the assembling of polyphenol-rich items from plants for their further use in pharmacological, food and cosmetic industry. The primary objective of this investigation is to conduct chemical profiling of leaves from two olive tree species: wild (Olea cuspidata Wall.) and cultivated (*Olea europaea* L.) from natural Saudi ecosystems. The extracts of leaves were obtained in ethanol in order to study and assess their phytochemical profiles, which included: 1) the polyphenols of leaves 2) the antioxidant activities of leaves 3) the quantify the most representative extracts compound using HPLC/MS 4) the antimicrobial activity of leaves.

(a) (b)

Figure 1. Olive trees and leaves (Wild (a) and cultivated (b)).

2. MATERIALS AND METHODS

2.1. Collection of Plant Material

The leaf samples of wild (Olea cuspidata Wall.) plant A and cultivated olive (*Olea europaea* L.) plant B were collected from Taif and Al-Baha Saudi Arabia respectively (Table 1). Leaves samples of wild and cultivated olive were collected from the mature young shoots of the tree canopy and placed individually in resalable plastic bags, labeled properly and then transported carefully to the laboratory in liquid nitrogen for further analysis. The leaves samples were kept in refrigerator at −80˚C until required [17]. The sampling locations were selected because they represent very different microclimatic conditions and allowed the sampling of cultivated and wild olive ancestries at the same time (Figure 1).

2.2. Preparation of Leaves Sample

The olive leaves were thoroughly cleaned and washed with tap water and then rinsed with distilled water of analytical grade. After chopping into small pieces, the rinsed olive leaves of collected samples were air-shade dried and finely powdered by crushing in electronic grinder [18].

Table 1. Quantitative analysis of compounds (mg/g^{-1} dry weight), carried out by HPLC ESI/MS-TOF, of the wild and cultivated leaves extract.

Compound	Area ×10^2			RT			µg/mL		
	STD	wild	cul	STD	wild	cul	STD	wild	cul
Chlorogenic acid	2968	2131	10,870	7.34	7.34	7.34	0.08	0.11	0.59
Daidzein	7951	ND	ND	12.93	ND	ND	0.08	ND	ND
Gallic acid	3084	471.6	878.5	3.85	3.88	3.87	0.08	0.02	0.05
Caffeic acid	30,460	46,440	134,500	8.04	8.02	8.03	0.08	0.24	0.71
Rutin	20,080	2,334,000	722,600	9.72	9.70	9.71	0.08	18.60	5.76
Coumaric acid	50,910	264,100	246,400	9.53	9.52	9.52	0.08	0.83	0.77
Vanillin	1799	1626	2423	9.57	9.55	9.56	0.08	0.14	0.22
Naringenin	189	20,490	25,900	15.05	14.98	14.99	0.08	17.35	21.93
Querectin	29,710	580,200	409,100	13.59	13.59	13.59	0.08	3.12	2.20
Ellagic acid	708.8	7949	ND	9.92	9.91	ND	0.08	0.02	ND
Hesperetin	6181	ND	ND	15.64	ND	ND	0.08	ND	ND
Myricetin	4039	344	282.4	11.72	11.70	11.71	0.08	0.01	0.01
Cinnamic acid	239.9	ND	ND	14.20	ND	ND	0.08	ND	ND
Methyl gallate	47,430	ND	ND	7.45	ND	ND	0.08	ND	ND
Kaempferol	2458	538.6	1903	15.36	15.35	15.35	0.08	0.04	0.12
Ferulic acid	1911	4087	8357	10.25	10.24	10.24	0.08	0.34	0.70
Syringic acid	571.3	396.4	579.5	8.41	8.39	8.39	0.08	0.11	0.16
Apigenin	145.4	1151	322.4	15.05	15.05	15.05	0.08	1.27	0.35
Catechin	1128	ND	ND	7.34	ND	ND	0.08	ND	ND
Luteolin	16,090	543,900	547,000	13.52	13.51	13.51	0.08	5.41	5.44
3.4-Dihydroxybenzoic acid	2426	21,590	21,180	5.72	5.71	5.71	0.08	1.42	1.40

2.3. Extraction

Samples of powdered material were extracted with ethanol, with a volume-to-weight ratio of 20:1, at room temperature for 72 h. Extractions were carried out under intensive stirring with magnetic stirrer. The supernatant was separated from the solid residue by filtering and centrifuging for 10 min at 5000 rpm. The solvent was removed, and the solid extract was totally dried under reduced pressure at 45˚C, using a rotary evaporator. The dry extracts stored at −20˚C until used for further analyses [18].

2.4. Total Polyphenol Content (TPC)

The TP contents in ethanol extract from olive organs were estimated, in triplicate, by the Folin-Ciocalteu method [19]. In the tube, 0.5 mL of Folin-Ciocalteu (Prolabo) reagent were added to 0.5 mL methanolic extract from leaves, pulp, and stone dry matter followed by 4 mL of sodium carbonate 1 M solution. The tubes were placed for 5 min at 45˚C in a water bath and then put in a cold-water bath. Absorbance was measured at 765 nm. Gallic acid (GA) was used to make the calibration curve. The TP contents were expressed as mg gallic acid equivalents per 100 g dry weight (mg GAE/100 g DW).

2.5. Determination of DPPH Free-Radical Scavenging Activity

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical assay was carried out spectrophotometrically. Aliquots (50 µL) of various plant extracts were added to 5 mL of 0.004% ethanol solution of DPPH. After incubating the samples for 30 min at room temperature, the absorbance was read against a blank at 517 nm. Ascorbic acid (AA) was used as reference standard and dissolved in ethanol to make the stock solution with the same concentration (1 mg/mL) [20].

$$I(\%) = (1 - AS/AC) \times 100 \quad (1)$$

where AC: is the absorbance of the control reaction (containing all reagents except the tested compound) and AS is the absorbance of the tested compound. The % of inhibition was determined from a graph plotting percentage inhibition against extract concentration. All experiments were performed in duplicate.

2.6. HPLC ESI/MS-TOF Analysis of Leaf Extracts

The analysis of the sample was performed using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) with an Exion LC AC system for separation and SCIEX Triple Quad 5500+ MS/MS system equipped with electrospray ionization (ESI) for detection [21].

Positive and negative MRM mode:

The separation was performed using ZORBAX SB-C18 Column (4.6 × 100 mm, 1.8 µm). The mobile phases consisted of two eluents A: 0.1% formic acid in water; B: acetonitrile (LC grade). The mobile phase was programmed as following, 2% B from 0 - 1 min, 2% - 60% B from 1 - 21 min, 60% B from 21 - 25 min, 2% B from 25.01 - 28 min. The flow rate was 0.8 mL/min and the injection volume was 3 µL. For MRM analysis of the selected polyphenols, positive and negative ionization modes were applied in the same run with the following parameters: curtain gas: 25 psi; Ion Spray voltage: 4500 and −4500 for positive and negative modes, respectively; source temperature: 400˚C; ion source gas 1 & 2 were 55 psi with a decluttering potential: 50; collision energy: 25; collision energy spread: 10 [22].

3. ANTIMICROBIAL ACTIVITY

3.1. Pathogenic Bacteria

The organisms studied were: Gram positive bacteria namely *Bacillus subtilis* NRRL B-543 and Staphylococcus aureus; NRRL B-313, Gram negative bacteria Escherichia coli; NRRL B-210, and Pseudomonas aeruginosa NRRL B23 27853 pathogenic yeast Candida albicans NRRL Y-477 pathogenic fungi Aspergillus niger NRRL-3 and Aspergillus flavus ATCC 16883. These microorganisms were obtained from Natural Research center, Department of Chemistry of Natural and Microbial product Cairo Egypt, and were

grown and maintained in on nutrient agar media (Difco 0001). To compere the antibacterial activity of leave extract against the human pathogenic bacteria Tetracycline (TE) and Novobiocin (NV), were utilized as positive control for bacteria with focus 30 μg/mL. The bacterial suspensions were balanced with saline to a convergence of 105 CFU/mL. (Neomycin (N) (30 μg) were used for fungi [23].

3.2. Well Diffusion Technique

Screening of antimicrobial activity was performed by well diffusion technique. The nutrient agar medium ((NA) for pathogenic bacteria and potato dextrose agar (PDA) for fungi were used. The plates were seeded with 0.1 mL of the standardized inoculums of each test organism. The inoculums were spread evenly over plates with glass spreader. The seeded plates were allowed to dry in the incubator at 37˚C for 20 minutes [24]. A standard cork borer of 8 mm was used to cut uniform wells on the surface of media and 100 μL of each peel extract was introduced in the wells. The inoculated plates were incubated at 30˚C - 37˚C for 24 - 96 hours and zone of inhibition was measured to the nearest millimeter (mm). The zone of inhibition produced by the plant extract was compared with control [25].

3.3. Statistical Analysis

The results were subjected to one-way ANOVA analysis, followed by the Tukey-HSD (honestly significant difference) post hoc test ($p < 0.05$). All data were reported as the mean_SD with at least three replications for each olive leaf sample. Statistical analyses were performed using GraphPad version 6.01 (GraphPad Software, San Diego, CA, USA).

4. RESULTS AND DISCUSSION

4.1. Morphological Parameters

The morphological boundaries of olive leaves were subjected to statistical analysis, which revealed significant disparities that represent the inconsistency between the two species. It was determined that the genotype and environmental variables influenced the morphological boundaries of olive trees [26] and they changed fundamentally among developed and wild olive trees [27, 28].

4.2. Total Phenolic Content (TPC) of Extracts

Folin-Ciocalteu reagent, Gallic acid was utilized as a standard compound and the complete phenols were communicated as milligrams of gallic corrosive per gram separate (mg GA/g remove). The estimations of total phenolic content depended on the acquired adjustment bend from investigations of gallic corrosive standard arrangements. For the pre-owned alignment bend: $R^2 = 0.9998$, condition: $y = 0.0091x + 0.0038$. The consequences of investigations and estimations are displayed in **Figure 2**. The phenolic content of concentrates changed because of various kinds of olive leaves went from 21.3 to 22.6 mg GA/gdw. A phenol extract from olive leaves (*Olea europaea* L.) with a substantial hydroxytyrosol concentration boosted the redox stability of diverse dietary lipids (spread, grease, and oil extracted from cod liver). Salta *et al.* [29] demonstrated a growing interest in the use of conventional cancer preventive medicines. Because of the geographical differences, it was hypothesized that the chemical components and functional properties of African wild olive leaves would differ from those of European olive leaves [30]. Furthermore, a study conducted on 25 and 80 cultivars during 2014/2015 and 2015/2016 seasons, respectively, revealed large amounts of various phenolic compositions such as p-HPEA-EDA (about 2931.1 $mg{\cdot}kg^{-1}$ during 2014/2015 season), 3,4-DHPEA-EA (about 3501.3 $mg{\cdot}kg^{-1}$ during 2015/2016 season), in addition to other phenol derivatives (p-HPEA-EA and 3,4-DHPEA-EDA) [31]. Surprisingly, these appear to be the first and most important attributes mentioned in the literature.

4.3. DPPH Radical Scavenging Activity

The antioxidant properties of leaf extracts were assessed using the DPPH method to detect their free

radical scavenging activity. The DPPH ranged from 71% to 57% for wild and cultivated leaf extracts, respectively. The difference between DPPH average values of both leaf extracts is shown in Figure 3.

The acquired information shows that wild olive leaves extricate has higher antioxidant prevention agent movement than developed. These outcomes are antioxidant with the all-out phenolic content information aside from ethanol extracted, where the developed leaves separate contain more elevated levels of total phenolic. The polyphenols' antioxidant activity is mostly attributable to their redox characteristics, which allow them to act as reducing agents, protons donors, and singlet oxygen quenchers, as well as having the potential to chelate metals. Many phytochemicals, such as ascorbic acid, flavonoids, α-tocopherol, β-carotene, and polyphenols, have been demonstrated to possess strong antioxidant activity [32]. The antioxidant effects of phenolic substances are noteworthy, and they continue to elicit extensive logical investigation. They also have been shown to be effective cell regeneration players and to have antitumor effects [33].

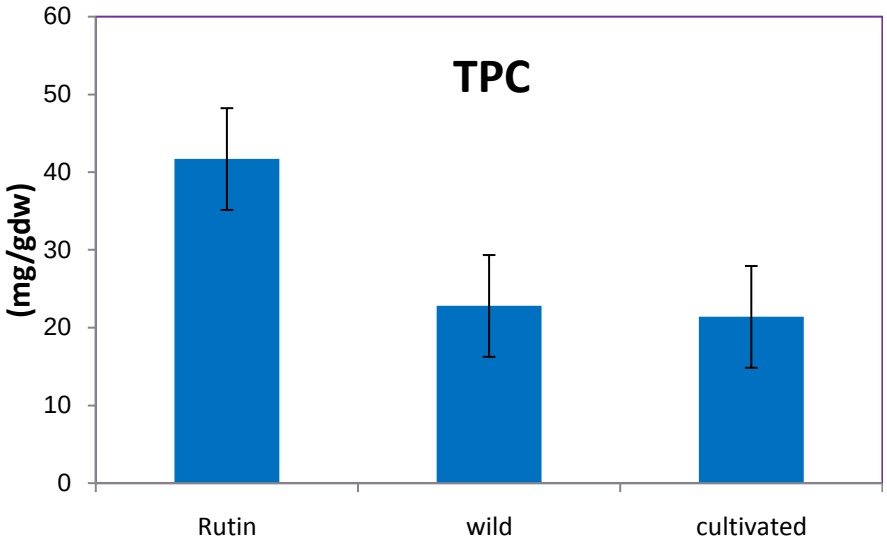


Figure 2. Total phenolic content (TPC) of wild and cultivated leaves extract.

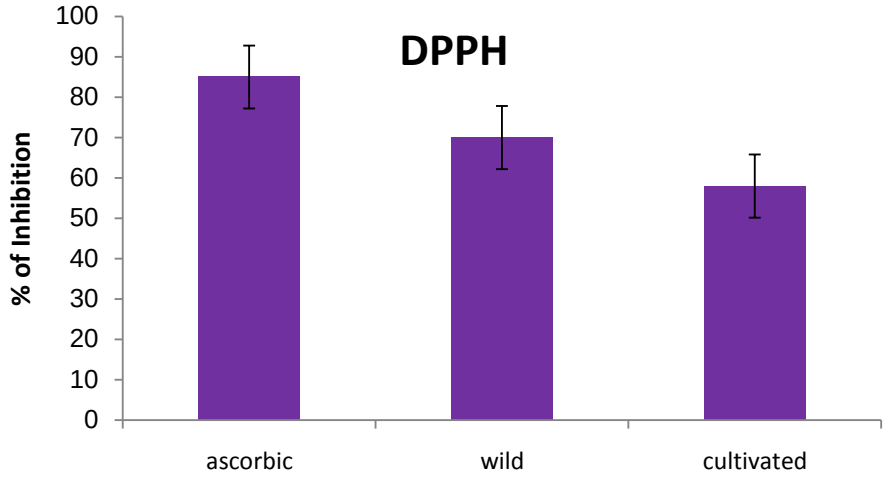


Figure 3. DPPH radical-scavenging activity of wild and cultivated leaves.

4.4. Extract

The phenolic compound can be dynamic as antioxidant agents by a few possible pathways. The most significant is probably going to be by free radical scavenging in which the phenol can break the free radical scavenging chain response. The presence of various substituents inside the spine design of phenols adjusts their cell reinforcement properties and their hydrogen-donating capacity. **Figure 3** shows a close association between phenolic content and antioxidant activity of leaf extracts. This is due to a large amount of phenolic compounds and their potent scavenging properties. The data also emphasises the importance of the synergistic activity of bioactive combinations in extracts, which is frequently more advantageous than a single restricted constituent [28, 33].

HPLC ESI/MS-TOF Analysis of Leaf Extracts

For quantitative determination of polyphenols in the obtained extracts, we used the HPLC method developed and validated by Fuad Al-Rimawi [21, 22]. Using HPLC ESI/MS-TOF, phenolic components were separated from the leaves of two olive cultivars and were categorized, yielding 21 distinct compounds. **Table 1** lists the phenolic compounds identified by bad ionization mode, along with references, retention times, peak areas, and quantities.

First, a sample of standard solution of polyphenols with appropriate concentration was subjected to analysis. Polyphenols were eluted within a retention time of 0 - 30 minutes, as shown by the chromatogram (**Figure 4**). HPLC analyses of dried olive leaf extracts were next conducted.

Two of the obtained chromatograms from the analysis of crude extract solutions (ethanolic extracts of wild and cultivated leaves are presented in **Figure 5** and **Figure 6** respectively. It is obvious that the major polyphenol in the extracts is naringenin, which appears at peaks 8 with concentrations of 21.93 and 17.35 μg/mL for cultivated and wild leave respectively, which retention time of 14.99 and 14.98 (**Table 1**). The second major peaks are 5 that appears in the chromatogram and corresponds to other phenolic compounds were identified as flavonoids, such as rutin that are present in the cultivated olive leaves at much less levels than wild (18.6 and 5.76) μg/mL with retention time 9.71 and 9.70 respectively. It is worth focusing on that rutin likewise exhibits numerous pharmacological exercises, for example, cytoprotective, cardioprotective, anti-carcinogenic and vasoprotective, while it is associated with Alzheimer and antiarthritic impacts [34]. In addition, The HPLC profile also shows Vanillin in peak 7 with retention time of 9.55 - 9.57. Also, coffic acid was detected at peak 4 with low concentrations in the wild than in cultivars (**Table 1**). Although coffic acid has been distinguished in olive leaf extract, barely any examinations have announced its evaluation [35]. Coffic acid has been studied in different plant species since it contributes to their distinctive flavour [36] and has health benefits [37]. Coffic acid has cell reinforcement potential, according to Conti *et al.* [38], and functions as an oral microbe inhibitor when combined with other components. Coffic acid has been linked to Pierce disease symptoms in grapes [39] and in Olea during *Xylella fastidiosa* infection [40]. These results were like Abdel-Aziz *et al.* [41] who reported that olive tree leaves have a high content of flavonoids especially, vanillic acid and caffeic acid in cultivated and wild. Flavonoids are one of the most abundant compounds in olive leaves [42]. Its benefits to human health have been widely recognized. It has antiviral, cardioprotective, and sedative properties, as well as protecting the membrane from lipid oxidation and preventing coronary disease. In addition, it lowers cholesterol level and hypertension in a multitude of medical conditions [43]. Soler-Rivas *et al.* [44] reported that, the aglycones apigenin, quercetin, kaempferol and hesperetin, were identified by reversed-phase HPLC.

Ellagic acid appeared in peak 10 for wild with retention time of 9.02 and disappear in cultivated leaves. On the other hand, Ferulic acid, Syringic acid, Gallic acid, Myricetine and Kaempferol are the minor phenolic compounds in both olive lives. Some phenolic compounds were not detected, such as Daidzein, Hespentin, Cinnamic acid, Methyl gallate and catechin. These results are mostly consistent with many other studies [45, 46]. The data in **Table 1** showed that peaks 9, 18, 20 and 21 maintained of the modulated values of flavonoids compounds, Quercitrin Apigenin, luteolin and 3,4-Dihydroxybenzoic acid with the same retention time 13.5, 15.05, 13.5 and 5 respectively, therefore these samples were selected to extract the phenolic compounds which will be used as antioxidant. According to literature, luteolin was detected

at high levels in the cultivars and wild [28]. Data raw olives are a promising wellspring of flavonoids and particularly luteolin glycoside isomers. This can likewise be affirmed by the high overflow of these glucosides found in all examples of our review. It ought to be accentuated that during the maturing phase of olives, a debasement of the glucosides is completed, prompting an expansion in the grouping of the luteolin moiety. Among flavonoids, luteolin and its glucosides show the most noteworthy cancer prevention agent movement with numerous helpful impacts on human wellbeing. Besides, late examinations present the potential enemy of COVID-19 properties of luteolin by restricting with a high partiality to similar locales of the fundamental protease of SARS-CoV-2 as the control particle [47].

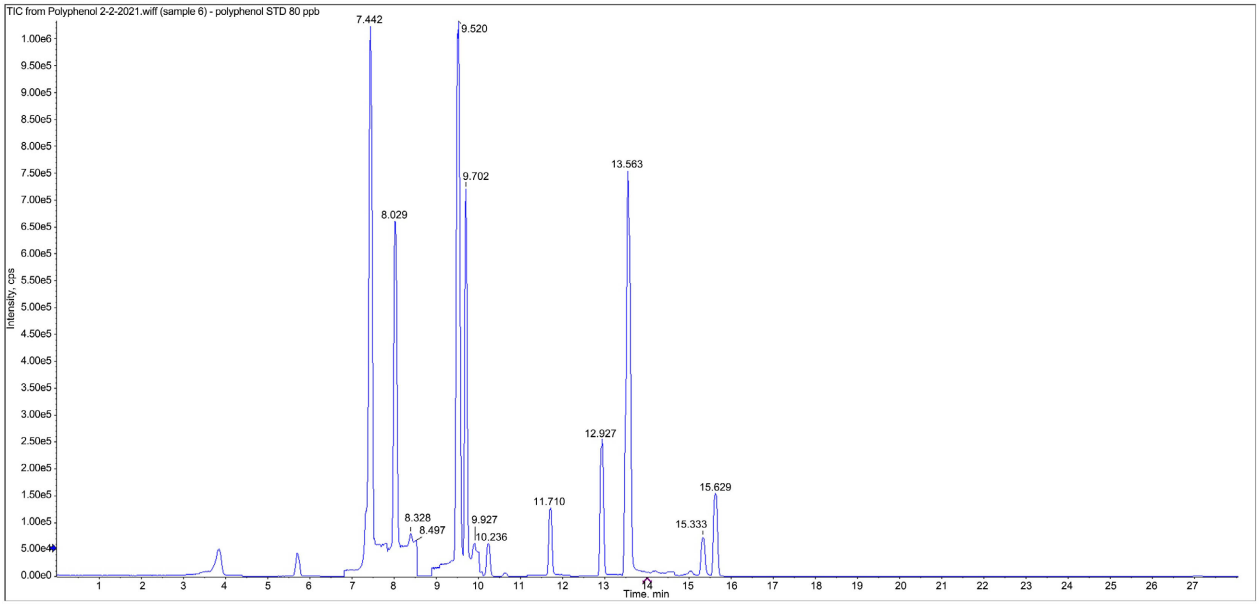


Figure 4. Chromatogram of stander compound classes detected: Base peak area of obtained by HPLC ESI/MS-TOFHPLC ESI/MS-TOF.

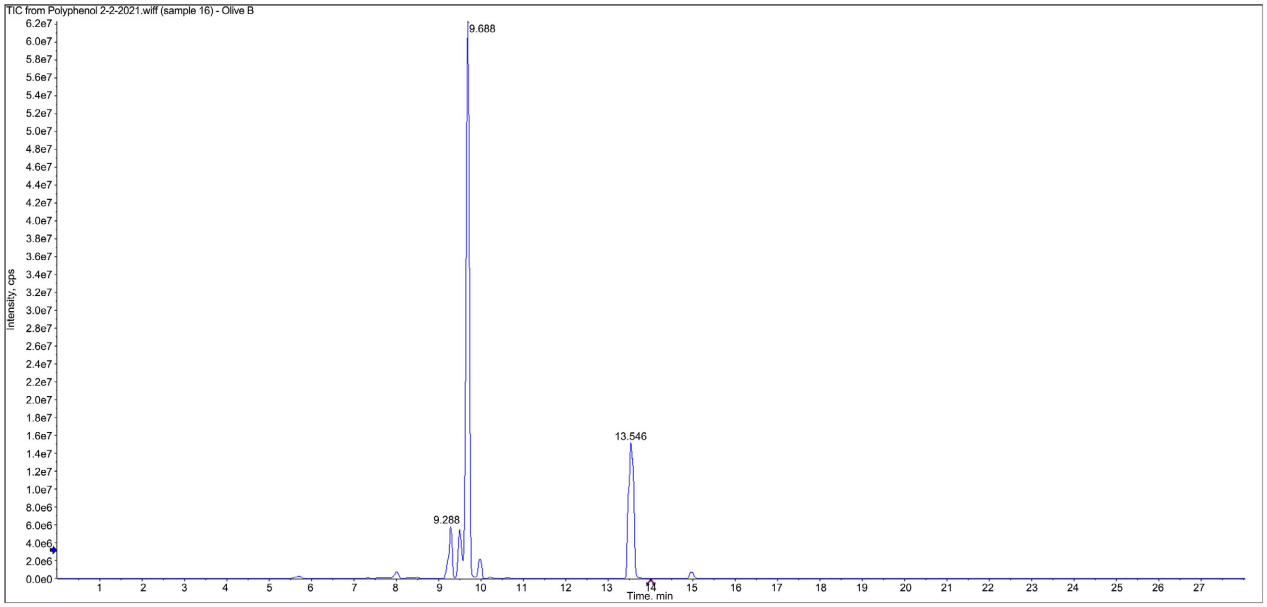


Figure 5. Chromatogram of wild olive leaf extract and classes of the compound detected: Base peak area obtained by HPLC ESI/MS-TOF.

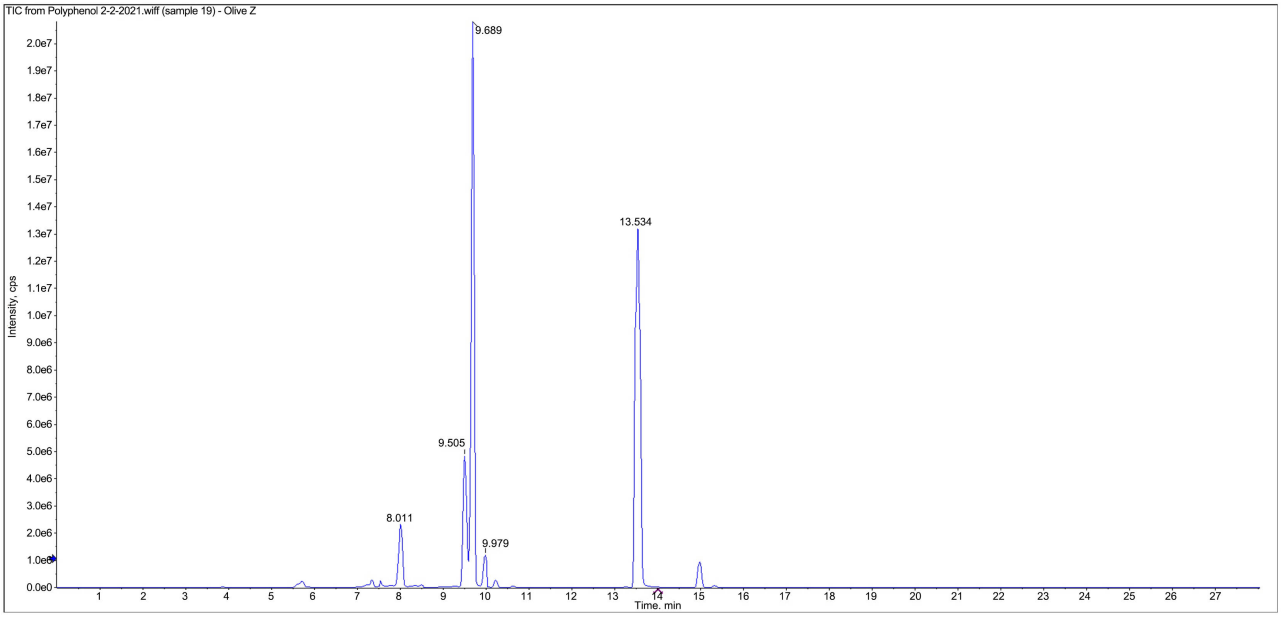


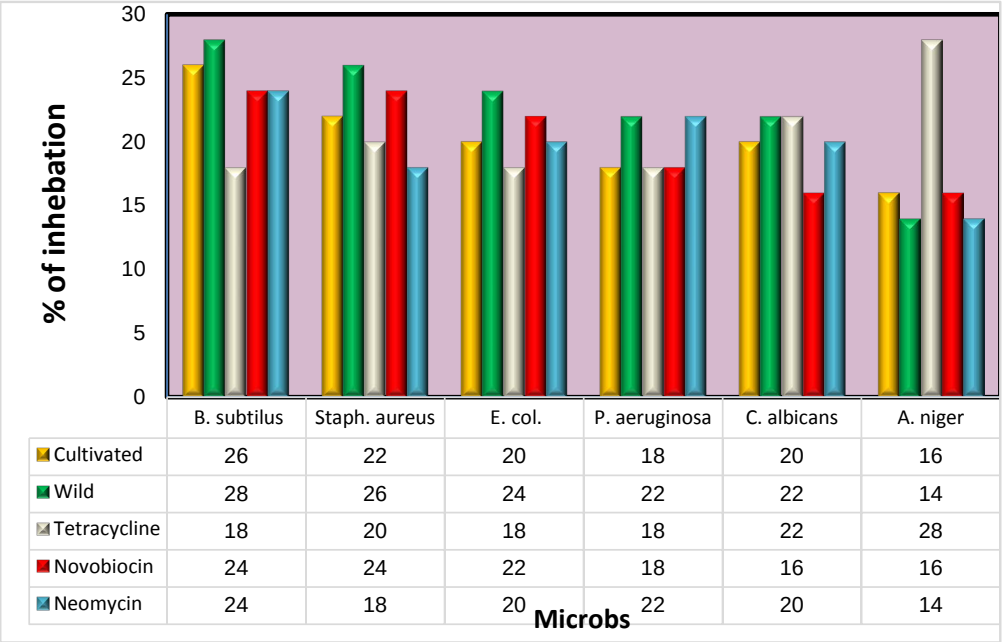
Figure 6. Chromatogram of cultivated olive leaf extract and classes of the compound detected: Base peak area obtained by HPLC ESI/MS-TOF.

4.5. Antimicrobial Activity

The antimicrobial activity of the two assortments leaves concentrates and standard antimicrobial agents were noticed obviously against the checked microbial strains (bacterial and fungi). **Figure 7** shows the antibacterial activity of wild and cultivated olive plant leaf extracts. Four bacterial strains (two Gram negative and two Gram positive) and two pathogenic fungal strains were tested for antimicrobial activity of olive plant leaf extracts against antibiotics such as Tetracycline (TE), Novobiocin (NV), and Neomycin (N) in this study. The antibacterial activity of the two types of olive leaf extracts (wild and cultivated) was compared to that of conventional antimicrobials. The results were determined by calculating the least zone of hindrance (ZOI), which was displayed in millimeters (mm). The ethanol extracts of the two species of olive leaves (wild and cultivated) revealed a lot of activity. As shown in **Figure 7**, the two species extracts had significant antibacterial activity against each of the pathogens tested.

The well diffusion method was developed for measurements of the minimum zone of inhibition (ZOI), which specified the maximum inhibition activity against *Bacillus subtilis*, with the highest inhibition zones (28 and 26 mm) for wild and cultivated leaf extracts, respectively, (**Figure 8**) that could be compared with its antibiotic counterpart; (NV) and (N) with inhibition zone of 24 mm. The lowest ZOI values (16 and 14 mm, respectively) against AN were produced by wild and cultivated olive leaf extracts, which were two times lower than the standard antibiotic (TE).

Due to microbial multi-drug resistance, phytochemicals are in desperate need of new antimicrobial treatments. Saponins are another important phytochemical component of plants that has antibacterial properties [48]. A few studies have accounted for the antimicrobial activities of olive leaf extracts. The antibacterial activity of olive leaf extracts against *Bacillus subtilis*, *Proteus vulgaris*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Citrobacter freundii*, and *Streptococcus pneumoniae* [49], in addition to the antibacterial properties of ethanolic extracts from wild and cultivated olive leaves in relation to the antimicrobial compounds used, were indeed evaluated. The findings of this study were also found to be innovative in comparison to other studies [49]. Alkaloids, tannins, and saponins are amongst the naturally occurring phytochemicals found in olive extracts that were shown to exhibit antibacterial action against known pathogens. Additionally, flavonoids are hydroxylated phenol-rich mixes that are believed to have antibacterial activity due to their complex interactions with extracellular



	B. subtilus	Staph. aureus	E. col.	P. aeruginosa	C. albicans	A. niger
Cultivated	26	22	20	18	20	16
Wild	28	26	24	22	22	14
Tetracycline	18	20	18	18	22	28
Novobiocin	24	24	22	18	16	16
Neomycin	24	18	20	22	20	14

Figure 7. The diameter of inhibition zone (mm) surrounding wild and cultivated leaves extract in presence of various microorganisms.

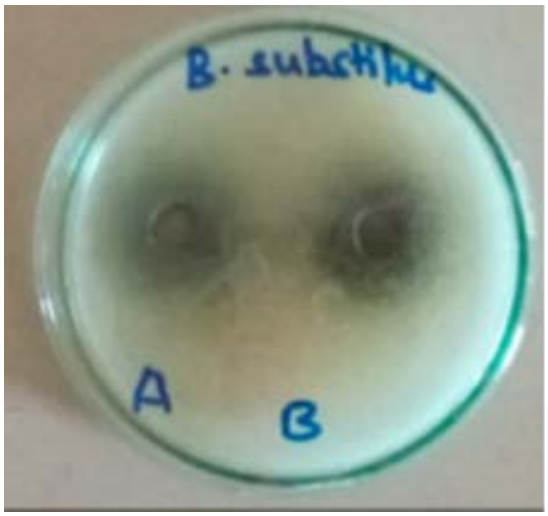


Figure 8. Agar plates containing zones of inhibition among the *Bacillus subtilis*, of ethanol extracts where (A) wild (B) cultivated of olive leaves.

and intracellular proteins, as well as the cellular structure of microbial species [50]. In comparison to hexane, ethanolic extracts of both wild and cultivated olive species had the highest antibacterial activity because they contained flavonoids and phenolic phytochemicals.

In addition, when wild and cultivated olive leaf extracts were tested for antibacterial efficacy against different bacteria, the lowest ZOI values (24, 20 and 22, 18 mm, respectively) were recorded against *E. coli* and *Pseudomonas aeruginosa*. The antibacterial activity of the used olive leaf extracts was found to be varied in terms of efficacy when compared to their counterpart antibiotics utilized in this investigation, with some bacterial strains exhibiting greater resistance and others showing more sensitivity to the plant extracts.

5. CONCLUSION

In conclusion, the screening of both wild and cultivated olive leaf extracts, especially wild species, revealed that they had strong restorative capabilities and might be used as viable and substantial medicinal sources. Furthermore, olive leaf extracts exhibited a better oxidative adjustment effect than pharmaceutical therapies that were commercially manufactured. Olive leaves can thus be regarded as a potential antimicrobial agent with a long history of use. However, more research on the phytochemical, molecular, and therapeutic properties of olive leaf extracts is needed to have a better understanding of the mechanisms that allow these extracts to be utilised as effective antibacterial agents.

FUTURE PERSPECTIVE

This entire study reveals that the explored plant is important to be investigated, and in a much better way, using various microbial strains, advanced apparatus, and any or all of the available ordinary and traditional approaches. The deposits of farming and food businesses address a difficult issue from a monetary and natural perspective, and accordingly taking advantage of such side-effects could prompt high worth added items to test the expected applications for human use.

CONFLICTS OF INTEREST

The author declares no conflicts of interest regarding the publication of this paper.

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