Journal of Food Measurement and Characterization
https://doi.org/10.1007/s11694-019-00310-5

ORIGINAL PAPER

Phytochemical composition, antioxidant and antimicrobial activities of leaves of *Olea europaea* wild variety

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Received: 1 March 2019 / Accepted: 18 October 2019
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Abstract

In the present study, three different leaf extracts of wild variety of *Olea europaea* were tested for their phytochemical composition, antioxidant and antimicrobial activities. The antimicrobial activities were determined using the well diffusion method. Phytochemical screening revealed that aqueous, ethanol and ethyl acetate extracts contained alkaloids, flavonoids, steroids, saponins, phenols and tannins while only ethyl acetate fraction was negative for glycosides. Ethanol extract of the plant was observed to have highest contents of alkaloids, flavonoids and phenols. The free radical reducing power assay, DPPH and hydrogen peroxide radical scavenging activities of ethanol extract of *O. europaea* showed a linear correlation with phenol contents. Ethanol extract was more effective against most of the pathogenic bacteria while aqueous and ethyl acetate extracts were found more potent against most of the fungal strains. The current study revealed that wild variety of *O. europaea* is as an efficient source of antioxidant and antimicrobial phytochemicals.

Keywords Antioxidant activity · Antibacterial activity · Antifungal activity · *Olea europaea* · Phytochemicals

Introduction

Medicinal plants are widely used for the treatment of different diseases due to the presence of biologically active constituents [1–3]. *Olea europaea* L. is an evergreen plant of the family Oleaceae, commonly known as olive. It is well recognized from ancient times, mostly found in the coastal zones of the Mediterranean regions. Olive has increased its importance throughout the time due to its high nutritional values and medicinal potentials. The medicinal properties of olive fruit, leaves, and oil are recognized as necessary parts of medication and diet [4–7].

Olive oil has protective effect on cancer and cardiovascular health due to the presence of various antioxidants. Similarly, the monounsaturated fatty acids and other bioactive functional components such as phospholipids, carotenoids, tocopherols and phenolic in olive oil have further increased its natural health benefits. Olive leaves extracts show antibacterial, antifungal and antioxidant properties [8, 9]. Because of its antimicrobial activities, olive can be effectively used for the treatment of infectious diseases. The therapeutic uses of olive (*O. europaea*) have been shown in traditional medications. It has been known that *O. europaea* reduces the high cholesterol, blood sugar and uric acid levels and has also been used for the treatment of diabetes, inflammation, diarrhea, hypertension, urinary tract and respiratory infections, stomach and intestinal diseases, hemorrhoids, rheumatism asthma, mouth cleanser and laxatives [7].

Several phenolics particularly oleuropein, iridoids and secoiridoids have been isolated from *O. europaea* [10, 11]. Oleuropein is one of the most essential chemical constituents of the *O. europaea* having broad medicinal activities including anti-inflammatory, anti-atherogenic, anticancer, antimicrobial and antiviral, hypolipidemic activities [6, 7, 12]. The chemical composition of olive and its oil depend upon different factors such as cultivar, ripeness strategies, environmental situations and the techniques used for processing [5, 7, 13, 14]. The present study aims to investigate the chemical composition and biological potentials of different

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Published online: 26 November 2019

leaf extracts of Pakistani wild variety of *O. europaea* for their phytochemical contents, antibacterial, antifungal and antioxidant activities.

Materials and methods

Collection and processing of plant sample

Olea europaea plant was collected from the local area of Dir district of Khyber Pakhtunkhwa, Pakistan. The plant was identified by the botanical taxonomist at the PCSIR Laboratories Complex Peshawar, Ministry of Science and Technology, Government of Pakistan. For reference, voucher specimens were preserved and stored in PCSIR laboratories.

Preparation of plant extracts

The leaves of wild variety of *O. europaea* were thoroughly cleaned, washed with tap water followed by rinsing with distilled water. After chopping into small pieces, the leaves were shade dried and finely powdered by crushing in the grinder. 100 g of finely ground olive leaves powder was extracted with 350 mL of distilled water, ethanol, and ethyl acetate, separately. The procedure was repeated three times to fully extract the leaves materials and all extracts were mixed. The rotary evaporator (40 °C) was used for the removal of solvents under vacuum [15, 16].

Phytochemical screening

Qualitative and quantitative analyses of phytochemicals

The qualitative tests for the presence/absence of phytochemicals were performed for alkaloids, glycosides, saponins, flavonoids, phenols/tannins, steroids and terpenoids while quantitative analysis was carried out for alkaloids, flavonoids and phenols [1, 2, 17–20]. Oleuropein was detected in the ethanol extract by thin layer chromatographic method, as described previously [7]. The spots were visualized by spraying with sulfovanillin and then heated at 100 °C for 10 min to obtain considerable coral pink coloration. Isoverbascoside (Merck, Germany) was used as reference standard.

FT-IR analysis

Functional groups and types of chemical bonds present in phytochemicals were identified by FT-IR spectroscopic analysis. For FT-IR spectral data, an appropriate quantity of the solvent extract was mixed with KBr (IR grade) and transferred to a sample cup of the diffuse reflectance accessory (DRS-8000 A). FT-IR spectral analysis was performed using FT-IR spectrometer (Prestige-21, Shimadzu, Japan). The FT-IR spectra were measured in the range of 4000 to 400 cm^{-1}.

Antioxidant activity

DPPH radical scavenging activity

2,2-Diphenyl-1,1-picryl hydrazyl (DPPH) solution (0.2 mM) was prepared using methanol. 1 mL DPPH solution was mixed with 2 mL of aqueous, ethanol and ethyl acetate extracts (1 and 2 mg mL^{-1}). The mixture was incubated at room temperature for 30 min in dark. The absorbance of the resultant solution was then measured at 517 nm against the reagent blank using UV–Vis spectrophotometer (UV-1602, Biotechnology Medical Services, USA). The DPPH radical scavenging activity was calculated using the following equation [18–20];

$$\text{DPPH radical scavenging activity (\%)}: \left[(A0 - A1)/A0\right] \times 100,$$

where A0 is the absorbance of the blank (solvent + DPPH) and A1 is the absorbance of the solvent extracts.

Reducing power assay

Reducing power assay of the plant samples was determined as described previously [19, 20]. Briefly, 1 mL of sample (1 and 2 mg mL^{-1}) was mixed with phosphate buffer (2.5 mL, pH 6.6) and potassium ferricyanide (2.5 mL, 1%) and was incubated at 50 °C for 20 min, followed by the addition of trichloroacetic acid (2.5 mL, 10%). The mixture was subjected to centrifugation and the upper layer was removed and mixed with distilled water (2.5 mL) and ferric chloride (0.5 mL, 0.1%). The optical density was measured at 700 nm using UV–Vis spectrophotometer. Ascorbic acid was used as a positive control.

Hydrogen peroxide radical scavenging activity

40 mM solution of hydrogen peroxide was prepared in phosphate buffer (pH 7.4). 0.1 mg mL^{-1} of the extract was added to hydrogen peroxide solution and absorbance was measured at 560 nm using UV–Vis spectrophotometer against blank solution containing phosphate buffer without hydrogen peroxide [21]. Ascorbic acid was used as a positive control. The percentage of hydrogen peroxide scavenging was calculated.

Antimicrobial assay

Antibacterial activity

In vitro antibacterial analysis was performed using disc diffusion method [19, 20]. All extracts were tested against nine bacterial strains, including three from Gram positive and

six from Gram negative groups (Table 3). About 15–20 mL of sterilized molten agar was poured on sterile glass petri dishes (100 mm) and allowed to solidify. Agar surface of each plate was streaked with sterile cotton swab containing bacterial strain. Paper discs of 6 mm size were impregnated in 6 µL of each extract dissolved in DMSO (2 µg μL^{-1}) and were placed on solidified agar plates at equal distance. DMSO was used as negative control while clarithromycin (5 µg μL^{-1}) and ciprofloxacin (5 µg μL^{-1}) were used as positive controls. The plates were incubated at 37 °C for 24 h. The antibacterial activity was measured as zone of inhibition (mm).

Antifungal activity

Antifungal activity of the *O. europaea* was also investigated by disc diffusion method [20–22]. Plant extracts were studied against six pathogenic fungal strains including *Aspergillus flavus*, *Aspergillus nigar*, *Aspergillus parasiticus*, *Fusarium solani*, *Candida albicans and Candida glaberata* (Table 5). The nutrient agar solution (yeast and mould, 13 g L^{-1}) was prepared in different conical flasks and then sterilized at 121 °C for 20 min, cooled and transferred to petri dishes. Fungal species were cultured on all the plates. 6 µL of extracts dissolved in DMSO (2 µg μL^{-1}) were absorbed by the filter paper discs and placed in the culture plates. The antifungal activity was measured after 72 h of incubation at 30 °C. DMSO was used as a negative control while commercially available antibiotics (fluconazole and clotrimazole, 5 µg μL^{-1} each), were used as positive controls. The antifungal activity was measured as zone of inhibition in mm.

Results and discussion

Qualitative phytochemical screening

The various phytochemicals screened include; alkaloids, glycosides, saponins, flavonoids, phenols/tannins, steroids and terpenoids. All the studied phytochemicals were detected in all extracts except glycosides in the ethyl acetate extract (Table 1). TLC analysis showed that oleuropein, a biological active compound, was present in *O. europaea* leaves extracts (Fig. 1). Kishikawa et al. studied ethanol extract of olive plant and confirmed the presence of oleuropein through HPLC, LC/MS analysis in leaves, stems and flowers [23]. Oleuropein is highly potent against various microorganisms and is also important cytoprotective against cisplatin-induced genotoxicity through the restoration of the antioxidant system of the renal hydroxy-20-deoxyguanosine [24, 25]. The presence of various phytochemicals were further confirmed by FT-IR analysis of the extracts. FT-IR is an important tool for identifying the types of chemical bonds (functional groups) present in compounds. The FT-IR spectrum of various leaves extracts of wild variety of *O. europaea* is shown in Fig. 2.

Aqueous extract

Aqueous extract of *O. europaea* showed characteristic bands at 2918 cm^{-1} indicating C–H stretching, 2848 cm^{-1} for C–H aldehyde, 1714 and 1683 cm^{-1} for carbonyl (C=O) group, 1361 cm^{-1} for C–H bending, 1182, 1165 and 1076 cm^{-1} indicating the presence of C–O group while 1033 cm^{-1} for C–H alkene oop bending.

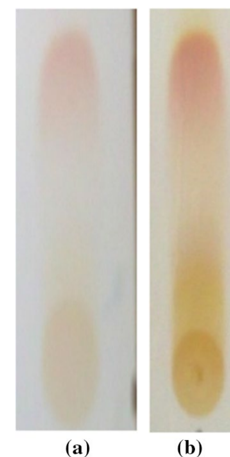

Fig. 1 TLC chromatograms showing the detection of oleuropein in **a** isoverbascoside (standard) solution and **b** ethanol extract of wild variety of *O. europaea* leaves

Table 1 Phytochemical screening of wild variety of *O. europaea* leaves

Extracts	Phytochemicals						
	Alkaloids	Glycosides	Saponins	Flavonoids	Phenols/tannins	Steroids	Terpenoids
Aqueous	+	++	+++	++	ND	+++	++
Ethanol	++	++	+++	+	+	+++	+++
Ethyl acetate	++	ND	++	+	++	+++	++

ND not detected, + photochemical detected, ++ photochemical detected at slightly higher concentration, +++ photochemical detected at higher concentration

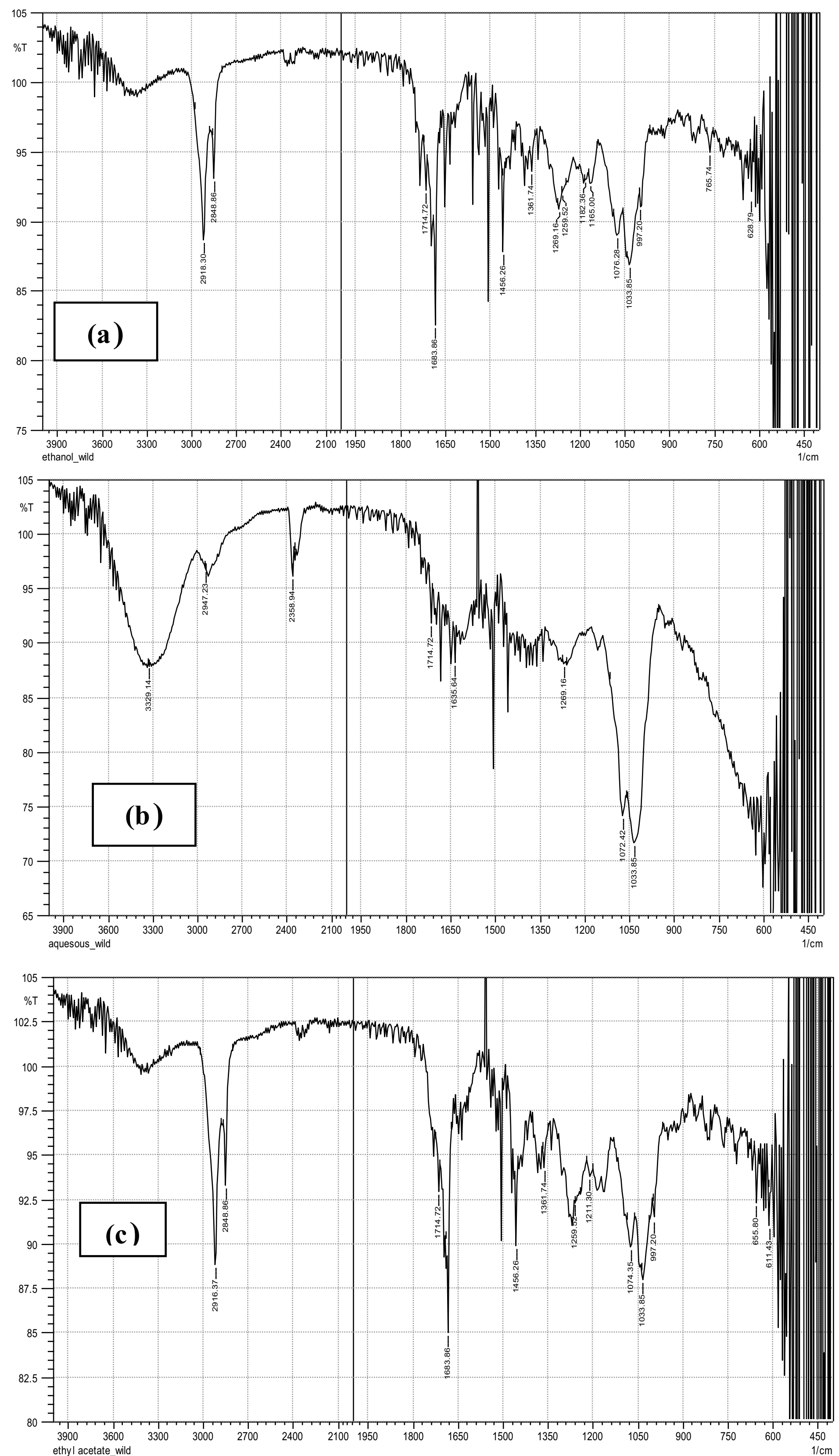

Fig. 2 FT-IR spectra of wild variety of *O. europaea* leaves*:* **a** aquas, **b** ethanol, **c** ethyl acetate

Ethanol extract

In the ethanol fraction the characteristic absorption bands were exhibited at 3329 cm^{-1} indicating O–H group, 2947 cm^{-1} for C–H stretching, 1714 cm^{-1} for C=O, 1635 cm^{-1} C=C, 1072 cm^{-1} C–O stretch, 1033 cm^{-1} C–H oop bend alkenes.

Ethyl acetate

Ethyl acetate extract showed bands at 3200 cm^{-1} for O–H stretch, 2916 cm^{-1} for C–H stretch, 2848 cm^{-1} for C–H aldehyde, 1714 cm^{-1} for C=O, 1456 and 1361 cm^{-1} for C–H bending, and 1033 for C–O stretch.

FT-IR analysis of leaves extracts in various solvents confirmed the presence of alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, etc.

Quantitative phytochemical analysis

Bioactive components including alkaloids, flavonoids and phenols were quantitatively analyzed in *O. europaea* leaves extracts (Table 2). The results showed that ethanol extract contained the highest quantity of alkaloids (12.50 ± 0.20%), and phenols (13.20 ± 0.22%) compared to aqueous ($P < 0.000$) and ethyl acetate ($P < 0.001$ and $P < 0.000$ respectively) extracts. Similarly ethyl acetate extract (7.30 ± 0.24%) showed higher quantity ($P < 0.001$) of alkaloids than the aqueous extract (1.30 ± 0.20%). Similarly, the quantity of flavonoids in both aqueous (14.0 ± 1.40%) and ethanol extracts (16.36 ± 0.20%) were significantly higher ($P < 0.000$) than ethyl acetate fraction (5.6 ± 0.12%).

A comparative study on total phenolic distribution has shown that ethanol extract of stem contains highest phenolic content compared to leaves and flowers [23]. Phenolic compounds have been well characterized for their peroxidation inhibition and free radicals scavenging activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties. They neutralize free radicals, quench singlet and triplet oxygen and decompose peroxides. These compounds play important roles in the control and treatment of various diseases including cardiovascular disorders, cancer, inflammatory diseases, and aging [26, 27]. Other phytochemicals such as flavonoids, anthocyanins, lignans etc. also contribute significantly towards antioxidant activity [28]. Studies indicate that phenolic composition of olive are primarily dominated by phenolic acids, phenolic alcohols, flavonoids and secoiridoids (e.g. oleuropein, ligstroside) [29]. Oleuropein is considered as the most abundant phenolic compound present in the olive leaves that gives bitter taste to olive and olive oil [30]. Various factors including cultivar, environmental conditions (e.g. region, climate and irrigation) and harvesting stage greatly affect phenolic profile of olive leaves [9, 31, 32].

Table 2 Quantitative phytochemicals analysis of wild variety of *O. europaea* leaves (dry weight basis)

Extracts	Phytochemicals (%)		
	Alkaloids	Flavonoids	Phenols
Aqueous	1.30 ± 0.20^a	14.0 ± 1.40^b	4.56 ± 1.36^a
Ethanol	12.50 ± 0.20^b	16.36 ± 0.20^b	13.20 ± 0.22^b
Ethyl acetate	7.30 ± 0.24^c	5.6 ± 0.12^c	6.20 ± 0.48^a

Mean values with different lowercase letters in the same column are significantly different ($P < 0.05$), ordered according to c > b > a

Antioxidant activity

DPPH free radical scavenging ability

DPPH free radical scavenging assay was determined for various extracts in a concentration dependent manner (Fig. 3). A linear co-relation was observed between phenolic content and antioxidant potential of *O. europaea* leaves extracts. The decreasing order of antioxidant activity in various fractions was ethanol > ethyl acetate > aqueous extract. Ethanol fraction showed 41.4 ± 1.01 and 78.3 ± 2.56%, ethyl acetate fraction showed 33.3 ± 1.40 and 63.4 ± 2.48% while aqueous fraction showed 28.6 ± 1.13 and 48.3 ± 2.06% DPPH scavenging potential at 1 and 2 mg mL^{-1} concentration of extracts, respectively. The DPPH free radical-scavenging data reveal that compounds within the leaves extracts are capable of scavenging free radicals via electron- or hydrogen donating mechanisms and thus should be able to prevent the initiation of deleterious free radical mediated chain reactions in vivo [33]. It has been observed that olive leaves reduce polyunsaturated fatty acids loss in soybean oil by inhibiting lipid peroxidation. Olive leaves extracts increase

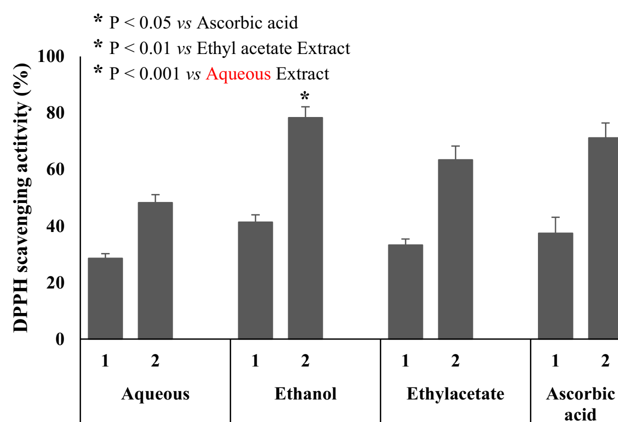

Fig. 3 DPPH scavenging activity of wild variety of *O. europaea* leaves

the availability of vitamin E and other phytochemicals that reduce the formation of oxidized compounds and hence prevent peroxidation [34].

Reducing power assay

Reducing power assay is an easy and fast screening method for determining the antioxidant activity. In this method, the reductive ability based on conversion of Fe^{3+} to Fe^{2+} is measured in the presence of sample. The yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of the sample. This change of colour is a measure of Fe^{2+} concentration. The reducing power of a sample serve as an indicator of its antioxidant potential [20]. The reducing ability of various extracts of *O. europaea* leaves is shown in Fig. 4. Ethanol extract of *O. europaea* showed highest reducing potential followed by ethyl acetate while aqueous fraction was least potent in terms of reducing power. The extracts showed an increase in reducing ability in a concentration dependent manner. At 1 mg mL^{-1}, the absorbance of plant extract for ethanol, ethyl acetate and aqueous fractions were 0.624, 0.342 and 0.042, respectively while at 2 mg mL^{-1} the absorbance values were 1.224, 0.592 and 0.106, respectively. The reducing ability of ethanol fraction was higher ($P<0.05$) than standard, vitamin C while the later was more active than ethyl acetate and aqueous fractions, indicating that ethanol fraction contains high content of various antioxidants.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is produced during normal metabolic process. It is a weak oxidizing agent that can bring about oxidation of essential thiol (SH) groups of enzymes and hence inactivate them. Studies have shown that inside the cells, hydrogen peroxide react with Fe^{2+} and possibly Cu^{2+} ions leading to formation of hydroxyl radicals that make it toxic [35]. Hydrogen peroxide scavenging activity of various extracts of *O. europaea* leaves is shown in Fig. 5. Like DPPH scavenging potential and reducing power assay, hydrogen peroxide scavenging ability of ethanol extract was significantly higher ($P<0.01$) than both ethyl acetate and aqueous fractions. Ethanol extract showed 40.25 and 76.37%, ethyl acetate 18.75, 37.25%, while aqueous extract showed 14 and 23% hydrogen peroxide scavenging potential for 1 and 2 mg mL^{-1} extracts, respectively. Hydrogen peroxide scavenging activity of various extracts of *O. europaea was* compared with vitamin C as a standard. Ethanol extract was more potent than vitamin C ($P<0.05$) while ethyl acetate and aqueous extract were less active than vitamin C in scavenging hydrogen peroxide radical.

Antimicrobial activities

Antibacterial activity

The antibacterial activity was determined for three Gram positive and six Gram negative bacterial strains. The sources of the bacterial cultures are given in Table 3. The antibacterial activities of various extracts of *O. europaea* are given in Table 4. The extracts showed low to high antibacterial activity against all the studied pathogenic Gram positive and Gram negative bacterial strains with zone of inhibition ranging from 13 ± 0.08 to 19 ± 0.81 mm were compared with the standard antibacterial drugs (23 ± 0.76 to 26 ± 1.40 mm). Different extracts showed different activities against different bacterial strains. For example, aqueous and ethyl acetate fractions were more potent ($P<0.01$) against *Salmonella typhi* (18 ± 1.25 and 19 ± 0.81 mm zone of inhibition respectively) compared to ethanol extract (15 ± 0.80 mm). Similarly the inhibitory activity of ethanol (19 ± 0.81 mm) and ethyl acetate (19 ± 0.07 mm) extracts against *Bacillus*

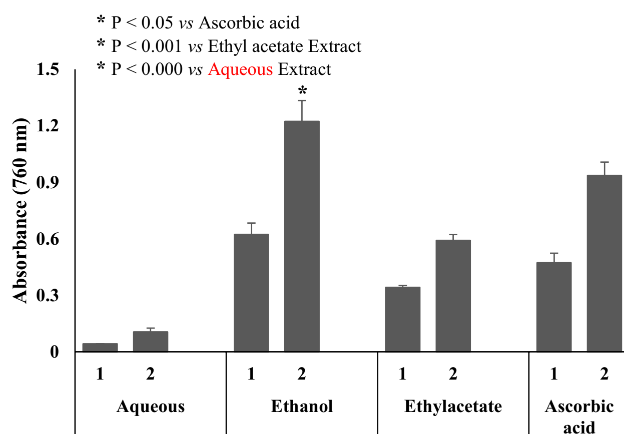

Fig. 4 Reducing power assay of wild variety of *O. europaea* leaves

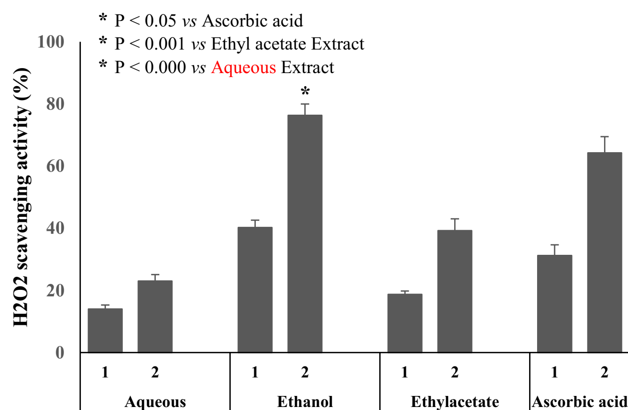

Fig. 5 Hydrogen peroxide scavenging activity of wild variety of *O. europaea* leaves

Table 3 Bacterial strains used for the antibacterial activity of wild variety of *O. europaea* leaves

Bacterial strains	Type	Source
Escherichia coli	Gram negative	ATCC25922
Salmonella typhi	Gram negative	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, Pakistan
Pseudomonas aeruginosa	Gram negative	ATCC9721
Klebsiella pneumonia	Gram negative	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Agrobacterium tumefaciens	Gram negative	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Erwinia carrotovora	Gram negative	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Staphylococcus aureus	Gram positive	ATCC6538
Bacillus subtillus	Gram-positive	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, Pakistan
Bacillus atrophus	Gram-positive	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan

Table 4 Antibacterial activity of wild variety of *O. europaea* leaves

Bacterial strains	Extracts			Standard drugs	
	Aqueous	Ethanol	Ethyl acetate	Clarithromycin	Ciprofloxacin
	Zone of inhibition (mm)				
Bacillus atnophus	17 ± 0.25^{a}	19 ± 0.81^{b}	19 ± 0.77^{b}	26 ± 5.40	–
Salmonella typhi	18 ± 1.25^{b}	15 ± 0.80^{a}	19 ± 0.81^{b}	–	23 ± 0.94
Escherichia coli	13 ± 1.95^{a}	15 ± 0.83^{a}	19 ± 0.81^{b}	–	24 ± 1.12
Agrobacterium tumefaciens	13 ± 0.08^{a}	15 ± 0.79^{b}	16 ± 1.63^{b}	–	23 ± 0.96
Pseudomonas aeruginosa	15 ± 0.80^{a}	14 ± 1.11^{a}	17 ± 1.50^{b}	–	25 ± 0.42
Staphylococcus aureus	17 ± 1.43^{b}	15 ± 0.08^{a}	17 ± 1.72^{b}	24 ± 1.02	–
Klebsiella pneumonia	15 ± 0.75^{a}	16 ± 1.52^{ab}	17 ± 1.35^{b}	–	24 ± 1.02
Bacillus subtillus	17 ± 1.25^{ab}	16 ± 1.73^{a}	18 ± 1.25^{b}	24 ± 1.55	–
Erwinia carrotovora	17 ± 1.25^{a}	17 ± 1.25^{a}	17 ± 1.25^{a}	–	25 ± 0.42

Mean values with different lowercase letters in the same row are significantly different (P < 0.05), ordered according to b > a

atnophus were significantly higher (P < 0.05) than aqueous extract (17 ± 0.25 mm). Ethyl acetate extract was potent against most of the tested bacteria, the lowest being observed against *Agrobacterium tumefaciens* (16 ± 1.63 mm) while the highest against *Escherichia coli* (19 ± 0.81 mm). The zone of inhibition of all extracts against *Erwinia carrotovora* were non-significantly different (P > 0.05). The data shows that there are some potential antibacterial agent(s) may be present in the solvent extracts of leaves of *O. europaea*. Owen et al. investigated that phenolic compounds present in cultivated variety of olive leaves extract were active against many bacteria including *B. cereus*, *E. coli*, *K. pneumoniae*, *S. aureus*, *S. typhi* and *V. parahaemolyticu* [36]. The antimicrobial potential of olive leaves extract against bacteria is strain and solvent dependent. Korukluoglu et al. reported that solvent type affect the phytochemicals distribution and antimicrobial activity against various bacteria. They observed that ethanolic extract of olive leaves were highly active against *E. coli* and *S. enteritidis*, while acetone extract showed highest antimicrobial efficiency against *S. thypimurium* [37]. Similarly, Markin et al. reported that 0.6% water extract of olive leaf significantly affects growth of *E. coli*, *P. aeruginosa*, *S. aureus* and *K. pneumonia* when exposed for short time, but the same aqueous extract is inactive against *B. subtilis* even at 20% (w/v) concentration [38]. Oleuropein, present in *O. europaea* leaves and fruits have been found to be active against many foodborne pathogens such as *Campylobacter jejuni*, *Helicobacter pylori* and *S. aureus* [30].

Antifungal activity

The tested fungal strains and the antifungal activity of various solvent extracts of *O. europaea* leaves are shown in Tables 5 and 6 respectively. Aqueous (13 ± 1.01 mm) and ethanolic (14 ± 0.21 mm) extracts showed higher inhibitory potential (P < 0.05) against *Aspergillus flavus* compared to ethyl acetate extract (11 ± 0.58 mm), while ethanol (11 ± 0.38 mm) and ethyl acetate (12 ± 0.45 mm)

Table 5 Fungal strains used for the antifungal activity of wild variety of *O. europaea* leaves

Fungal strains	Source
Aspergillus flavus	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Aspergillus nigar	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Aspergillus parasiticus	Clinical isolate obtained from Microbiology laboratory Quaid e Azam University Islamabad, Pakistan
Fusarium solani	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, Pakistan
Candida albicans	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, Pakistan
Candida glaberata	Clinical isolate obtained from Hayatabad Medical Complex Peshawar, Pakistan

Table 6 Antifungal activity of wild variety of *O. europaea* leaves

Fungal strains	Extracts			Standard drugs	
	Aqueous	Ethanol	Ethyl acetate	Fluconazole	Clotrimazole
	Zone of inhibition (mm)				
Aspergillus flavus	13 ± 1.01^{b}	14 ± 0.21^{b}	11 ± 0.58^{a}	–	21 ± 2.23
Aspergillus nigar	9 ± 0.36^{a}	11 ± 0.38^{b}	12 ± 0.45^{b}	22 ± 2.86	–
Aspergillus parasiticus	12 ± 0.17^{a}	13 ± 0.74^{a}	12 ± 0.61^{a}	19 ± 1.57	–
Fusarium solani	11 ± 0.56^{a}	11 ± 0.83^{a}	10 ± 0.33^{a}	–	20 ± 0.62
Candida albicans	10 ± 0.82^{a}	10 ± 0.71^{a}	12 ± 1.03^{b}	–	19 ± 1.02
Candida glaberata	11 ± 0.65^{a}	11 ± 0.82^{a}	10 ± 0.63^{a}	–	21 ± 0.81

Mean values with different lowercase letters in the same row are significantly different ($P < 0.05$), ordered according to b > a

fractions were more active ($P < 0.05$) in inhibiting the growth of *Aspergillus nigar* than aqueous fraction (9 ± 0.36 mm). All extracts showed nearly same activities ($P > 0.05$) against *Aspergillus parasiticus*, *Fusarium solani, and Candida glaberata.* Fluconazole and clotrimazole were used as standard drugs (positive controls). All extracts showed lower activities than standard drugs. A previous study has shown that acetone and ethyl acetate extracts of olive leaf possess various degrees of activities against various fungal strains including *Saccharomyces cerevisiae* ATCC 9763, *Schizosaccharomyces pombe*, *Saccharomyces uvarum*, *Candida oleophila*, *Metschnikowia fructicola* and *Kloeckera apiculate* while water fraction was found to be least active against the studied fungi. Moreover, *S. cerevisiae* ATCC 9763 was the most resistant among the yeasts [39].

Because of its high and diverse antimicrobial potential, olive leaves extract is considered as important nutraceutical taken either as liquid or capsules [40]. The antimicrobial potential of *O. europaea* L. is mainly attributed to the presence of on secoiridoid compounds and their derivatives including secoiridoids oleuropein, demethyloleuropein and ligstroside. These predominant phenolic compounds in *O. europaea* L. are exclusive to Oleaceae family [41]. They accumulate in fruit and leaf during maturation and act as defense molecules various pathogens.

Conclusion

The present study shows that leaves of wild variety of *O. europaea* possess different phytochemical components which contribute significantly towards the antioxidant potential and activities against some bacterial and fungal species. It would be of great interest if further research is carried out for the isolation of the biologically active constituents in purified form from this variety.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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