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ORIGINAL ARTICLE

Wild olive fruits: Phenolics profiling, antioxidants, antimicrobial, thrombolytic and haemolytic activities

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Abstract In this study, wild olive fruits were evaluated for the occurrence of phenolic antioxidant components and valuable nutrients which are distributed wildly in Soon valley of Pakistan. The shade-dried fruits of wild olive were extracted using different solvents to recover phenolic antioxidants. The highest concentration of extractable antioxidant components was recovered from tested fruits using aqueous ethanol compared to other solvents used. Crude concentrated extracts (CCEs) and phenolic rich fractions (PRFs) of tested fruits using hydroxyethanol were found to contain higher amount of total phenolic compounds and total flavonoid compounds along with superior biological attributes. According to ICP-OES analysis, potassium (17.96 g/kg) was the dominant macro element among other identified twenty-five minerals. The tested wild olive fruits juice was

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found to contain individual natural sugars including galactose (4.92 g/100 g dry weight), sucrose (2.75 g/100 g dry weight), glucose (0.73 g/100 g dry weight); and succinic acid (8.80 mg/100 g of dry matter) as major organic acid when analyzed on HPLC. Oleic acid (47.41 %) was the major monounsaturated fatty acid in the oil extracted from tested fruits. The concentration of phenolic antioxidants and biological activities vary significantly ($p < 0.05$) among extracting systems used. A strong correlation was also recorded among total phenolic (TP), total flavonoids (TF) and biological attributes of tested wild olive fruits. The results of this study explored wild olive fruits as a propitious source of natural phenolic components and valuable nutrients which reveal its potential use in the development of functional food and nutra-pharmaceuticals.

1. Introduction

Lipid oxidation is a major problem in food industry due to its adverse effects including rancidity and off-flavor. Due to its detrimental impact, different food ingredients have been reported to lose its organoleptic and nutrition value. A wide range of ailments including aging, metabolic syndromes, inflammations, cardiovascular problems and different types of cancers in various biological systems have been directly linked to occurrence of reactive oxygen species (ROS) and free radicals in them. Different parts of plants have been served as propitious source of food, fuel and folk medicine (F. Anwar et al., 2013). Medicinal benefits of plants have been ascribed to the presence of phytochemicals such as polyphenols, phytosterols, alkaloids and terpenoids in it. Phenolic antioxidant compounds have been documented to express multiple medicinal attributes including reduced risk of metabolic disorders, certain types of cancers and inflammation (Afata et al., 2022; Chen et al., 2022; Prasathkumar et al., 2021).

As a majority of conventional food plants are available with the occurrence of a variety of potent bioactives and high-value minerals for maintenance and regulation of bodily processes, but huge reserves of under-utilized wild plants are required to be explored for various bioactives and essential phytonutrients in order to meet the challenges of food security with increasing population and prevalence of different public health concerns. The fruits of different wild plants have been utilized in folk medicine systems of different civilizations (Shahidi, 2009).

Pakistan has been blessed with vast resources of different flora with medicinal importance which are available for biological prospecting. A variety of wild plant species from Pakistan have been consumed in folk medication system due to the occurrence of high-value nutrients and potent bioactive components (F. Anwar et al., 2013). Like other potential plant matrices, the fruits of wild olive can be explored for the occurrence of valuable plant-based bioactive compounds to produce nutraceuticals and functional foods with therapeutic properties. The Olive is one of the most prevalent fruit trees that is naturally distributed and extensively cultivated in different regions of world with moderate weather conditions to produce high quality of edible oil. Due to the presence of valuable phytonutrients and potent phenolic antioxidant components, the fruits of olive and its derived foods are documented as important ingredients for the formulation of food products with wide range of health benefits (Benavente-garcôâa et al., 2000; Hamid et al., 2022; Hannachi et al., 2020; Lins et al., 2018).

Due to a variety of biological attributes, the fruits of under-utilized wild plants are required to be processed for bioprospecting. These biological properties have been ascribed to the presence of different bioactives including phenolic antioxidant in various parts of wild plants. So, this study was framed to appraise the fruits of wild olive, native to Soon valley of Pakistan, for valuable phytonutrients, potent phenolic antioxidants and biological potential for the development of nutrapharmaceuticals and functional foods. This is a first report on evaluation of wild olive fruits from Soon valley of Pakistan for the presence of valuable nutrients and potent phenolic antioxidants.

2. Material and methods

2.1. Extraction and fractionation

The fully ripened fruits were obtained from olive (*Olea ferruginea* Royle) plants which were naturally grown in Soon valley of Punjab, Pakistan where weather conditions are characterized by annual precipitation (50 cm) with minimum and maximum average temperature of 1 °C and 36 °C, respectively. After authentication (voucher No 250-bot-21) by a Taxonomist, Department of Botany, Govt College University Faisalabad, samples were washed with distilled-water and dried under shade to store in -20 °C freezer for analyses. The preserved samples were minced in grinding mill (Tector–Cemotec 1090, Hognas, Sweden) and then soaked in various solvents including hydroxymethanol (80: 20, methanol: water), absolute methanol (100%), hydroxyethanol (80: 20, ethanol: water), absolute ethanol (100%), hydroxyacetone (80: 20, acetone: water) and absolute acetone (100%) for five days, followed by filtering using filter paper (Whatman, 8μ) to remove insoluble part. The excessive amount of solvent was recovered by rotary evaporator (EYELA, Tokyo, Japan) to get crude concentrated extracts (CCEs). According to Maheshwari et al., (2011), fractionation of CCEs was performed to produce phenolic rich fraction (PRFs). These CCEs and PRFs were stored at -20 °C.

2.2. Total phenolics (TP) in CCEs/PRFs

The content of TP in tested fruits were estimated following a method described by Ahmad et al., (2011). Briefly, accurately weighed (1mg/mL) of CCEs/PRFs was taken in a conical flask followed by addition of deionized water (7.5mL), Folin-Ciocalteu reagent (0.5 mL) and sodium carbonate (20% (w/v); 1.5 mL) to react in water bath at 40°C for 20 min. The final reaction mixture was cooled to ambient temperature whose optical density (OD) was recorded at 755 nm using spectrophotometer. The TP content was presented as Gallic Acid Equivalents (GAE) g/100g dry matter with the help of gallic acid standard curve.

2.3. Total flavonoids (TF) in CCEs/PRFs

The amount of TF in ripened wild olive fruits was quantified following a protocol described by Ahmad et al., (2011). Briefly, accurately weighed (100 mg/mL) of CCEs/PRFs was taken in conical flask followed by the addition of 5.0 mL distilled H_2O (for dilution) and sodium nitrite (5%; 0.3 mL). consequently, resulting mixture was incubated to mix with aluminum chloride (10%; 0.6 mL) and sodium hydroxide (1.0 *M*; 2 mL). The absorbance of final mixture was recorded at 510 nm. TF was calculated as catechin equivalents (CE) g/100g dry matter with the help of catechin calibration curve.

2.4. 2, 2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging analysis

This analysis was performed to assess the ability of CCEs/PRFs to scavenge DPPH radical (Tepe et al., 2005). It is expressed as IC_{50} value. DPPH solution (0.004%; 5 mL) was prepared in methanol and then reacted with each concentration of (0.10 to 5.0 mg/mL) and followed by incubation for 30 min in ambient conditions. Absorption of each sample was taken at 517 nm to estimate inhibition potential (Eq. (1)).

$$I(\%) = \left[\frac{A_b}{A_s}\right] \times 100 \quad (1)$$

Where, A_b and A_s are absorbance values of blank and sample, respectively, while I is percentage inhibition.

2.5. Reducing power of CCEs/PRFs

A method developed by Yen et al. (2000) was used to assess reducing power of CCEs/PRFs. Briefly, sodium phosphate buffer (0.2 M, pH 6.6), solution (1.0%) of $C_6N_6FeK_3$ and CCEs/PRFs (5-20 mg) were mixed in equal volume to incubate (50 °C) for 20 min. Then trichloroacetic acid (10%; 5 mL) was added to the resulting solution which was centrifuged (980 *g* ;10 min) using CHM-17, Tokyo, Japan) to recover supernatant (2.5 mL), followed by dilution using distilled H_2O (2.5 mL) and mixed with 0.1% iron (III) chloride (0.5 mL). The Absorption of the resulting reaction mixture was observed at 700 nm.

2.6. Ability of CCEs/PRFs to inhibit the process of peroxidation

CCEs/PRFs were evaluated for their potential to inhibit the process of peroxidation in linoleic acid (Ahmad et al., (2011)). For sample preparation, ten milliliter of sodium phosphate buffer (pH = 7, 0.2 M) was taken in conical flask followed by the addition of 5 mL distilled H 2O, linoleic acid (0.13 mL), ethanol (99.8%; 10 mL) and CCEs/PRFs (5 mg) to incubate at 40°C. Following Yen et al., (2000), sample solution (0.2 mL) was reacted with ten milliliter of aqueous ethanol (75%), 0.2 mL of NH_4SCN solution (30 %), and 0.2 mL of $FeCl_2$ (20 m M in 3.5% HCl) to record its absorption at 500 nm. Butylated hydroxytoluene and ascorbic acid were run as positive control. Inhibition of peroxidation was estimated by (Eq. (2)).

$$Inhibition\ (\%) = 100 - \left[\frac{A_s}{A_c}\right] - 100 \quad (2)$$

Where, A_s and A_c are the OD values of sample and control at 350 h.

2.7. Antimicrobial activity

According to (NCCLS, 1999, 1997), various CCEs/PRFs of tested fruits were evaluated for their antimicrobial potential against different fungal and bacterial strains by measuring the zone of inhibition and minimal inhibitory concentration (MIC). Fungal strains (*Aspergilus niger, Staphylococcus aureus and Aspergilus flavus*) were cultivated at 29 °C on potato dextrose agar while bacterial strains (*Fusarium oxysporum, Bacillus cereus and Escerichia coli*) were cultured on nutrient agar at 37 °C to get optimum growth. Fungal and bacterial broth cultures were developed in test tubes to achieve 10^4 cfu/mL and 10^8 cfu/mL. Filter paper discs of 6 mm diameter were sterilized. Each fungal and bacterial broth (100 µL) was applied on separate agar plates of their respective growth media followed by placing of soaked discs in CCEs/PRFs (100 mg/mL). Positive controls including fluconazole (for fungus) and rifamycin (for bacteria), and negative control (without CCE/PRF) were also executed with similar conditions of experiment.

In order to determine MIC, fungal and bacterial strains were cultured in sabouraud dextrose broth (SDB) and nutrient broth (NB) mixed with Tween 80. Under similar experimental conditions, growth control (NB + Tween 80), positive controls (tested microorganisms) and sterility control (CCE/PRF + NB + Tween 80) were also processed, while standards fungicidal (fluconazole) and bactericidal (rifamycin) drugs were also applied along with samples. The concentration of CCE/PRF with minimum or no growth of microorganism was labelled as MIC.

2.8. Inhibition of biofilm formation

According to Stepanović et al. 2000, CCEs/PRFs of tested fruits were appraised for their potential to inhibit the formation of biofilm caused by *E.coli* and *S. aureus* which were cultured in nutrient broth to achieve 10^9 CFU/mL. Briefly, different concentration (2.5 and 5.0 µg) of samples were solubilized in DMSO in wells of microtiter plate (96 well plate) followed by the addition of nutrient broth (100 µL) for microbial growth. This microtiter plate was inoculated with 20 µL of different bacterial broth. Under similar conditions of experiment, positive control (rifampicin) and negative control (bacterial broth) were also processed. After incubating (37 °C), control and sample wells were rinsed using autoclaved phosphate buffer (pH: 7.2; 220 µL) and aqueous methanol (99%, 220 µL) followed by air-drying. Staining of each well of microtiter plates was done using 220 µL of crystal violet solution (50 %) followed by washing by distilled water and resolubilizing in 220 µL of glacial acetic acid (33%). OD of each well in microtiter was taken at 630 nm. Biofilm inhibition (%) was calculated (Eq. (3)).

$$\text{Inhibition}(\%) = 100 - \left[\frac{OD_{\text{sample at 630 nm}}}{OD_{\text{control at 630 nm}}}\right] \quad (3)$$

2.9. Haemolytic potential of CCEs/PRFs

Haemolytic activity of CCEs/PRFs, derived from wild olive fruits, was determined by following a method (Yang et al., 2005). Briefly, cell suspension and different concentrations (125, 250, 500 and 1000 μg/ml) of plant extract was mixed in equal volume and placed at 37°C for half an hour, followed by centrifugation at 1500 rpm for 10 min. The recovered supernatant was processed for estimation of free hemoglobin at 540 nm. Distilled water and phosphate buffer saline were processed as hemolytic controls under similar conditions of experiment. Hemolysis (%) was estimated as Eq. (4).

$$\text{Hemolysis}(\%) = \left[\frac{A_t - A_n}{A_c - A_n}\right] \times 100 \quad (4)$$

Where A_c, A_n and A_t represents OD value of control (water), control (saline) and sample.

2.10. Thrombolytic potential of CCEs/PRFs

According to the reported method Prasad et al. (2006), CCEs/PRFs of tested fruits were appraised for thrombolytic potential. Concisely, various concentration CCEs/PRFs of tested fruits were separately mixed with blood sample (0.5 ml) at 37°C using water bath. Normal saline and streptokinase were also processed as blank and positive control, respectively. All samples and controls were run under similar experimental conditions.

2.11. Estimation of minerals in wild olive fruits

Inductively coupled plasma-optical emission spectroscopy (ICP-OES) was used to estimate mineral content in tested fruits following a method (Link et al., 1998). Hydrochloric acid and nitric acids (3:1) were employed to digest the samples at 182 °C for 9.5 min using microwave oven (USEPA method, 3051A), followed by cooling, filtration, centrifugation and dilution with water to analyze using ICP-OES. Pure water (18 MΩ cm) was utilized for washing, dilution, preparation of standards and samples.

2.12. Analysis of organic acids and HPLC conditions

Wild olive fruits were evaluated for the occurrence of organic acids following a method reported by Mahmood et al., 2012. Briefly, 10 g of fresh fruit aliquot was blended in 20 mL of distilled water to produce juice which was centrifuged at 3000g for 10 min followed by filtration through (0.25 μm) to get pure juice by removing suspended debris.

Organic acids were detected in fresh fruit's juice using HPLC (Varian Pro star, USA) equipped with UV-VIS detector. For the separation of organic acid, lichrospher column (RP-C18) was used while 0.001 N H_2SO_4 was selected as mobile phase whose flow rate was adjusted at 1.0 mL/min to detect different organic acids were at 230 nm. Various standards of organic acids including citric, gluconic, acetic, malic, oxalic and succinic acids were used to construct calibration curve which was used for quantitative analysis of sample for organic acids.

2.13. Analysis of natural sugars and HPLC conditions

Wild olive fruits were evaluated for the occurrence of natural sugars following a method reported by Mahmood et al., (2012). Briefly, 10 g of fresh aliquot of tested fruit sample was blended in 20 mL of distilled water to produce juice which was centrifuged at 3000g for 10 min followed by filtration through (0.25 μm) to get pure juice by removing suspended debris. Natural sugars were detected in fresh fruit juice using HPLC (Shimadzu, Japan) equipped with refractive index detector (RID-10A). For the separation of natural sugars, lichrospher column (R-100) was used and the flow rate of mobile phase (acetonitrile) was optimized to 0.6 mL/min to detect different organic acids. Natural sugars in tested wild fruits were detected and quantified by a comparison with standards.

2.14. Fatty acid composition and GC-FID conditions

The recovered oil from wild olive fruits was *trans*-esterified into FAMEs using a method reported by Farooq Anwar et al. (2008). Recovered oil (30 mg) was thoroughly mixed with potassium (sodium) methoxide (0.5 M; 5 mL). This mixture was incubated at 50 °C for 2 h in which samples were subjected to vortex after 3–4 mins to get monophasic system. Resulting solution was kept at room temperature for a while then allowed to react with distilled water (3 mL), isooctane (3 mL) and glacial acetic acid (0.3 mL). The final mixture was subjected to spin at 2500 rpm for 2 min to separate organic layer (FAMEs).

The recovered FAMEs were then analyzed on GC (Agilent Technologies, USA) coupled with FID (flame ionization detector) and capillary column (Zebron™ ZB-5, UK). Initial temperature of column oven was adjusted to 70 °C for 3 min that was further enhanced to 200 °C @ 9 °C/min. Then, the oven temperature was achieved to 260 °C for 40 min and carrier gas (N_2) flow rate was 2 mL/min. The injector and detector temperature were 240 °C and 260 °C, respectively. The fatty acids were identified by comparing with standards.

3. Results and discussion

3.1. Extraction yield

Pure solvents and their aqueous mixtures were used to extract antioxidant bioactive components of different polarities from the wild olive fruits. Therefore, various extracting solvents including absolute methanol and its aqueous mixture (80:20, methanol: water), absolute ethanol and its aqueous mixture (80:20, ethanol: water), absolute acetone and its aqueous mixture (80:20, acetone: water) were used to recover plant bioactives and their extraction yield (g/100g) of CCEs was presented in Table 1. The yield of extractable antioxidant components, recovered from tested the fruits of wild olive, varied considerably from 23.10 g/100g to 48.30 g/100g of dry matter. The lowest CCE yield (23.10%) was recorded using absolute acetone while aqueous ethanol recovered the highest CCE yield (48.30%) from the fruits of wild olive.

The antioxidant capacity of CCEs, recovered from wild olive fruits, was affected due the presence of impurities like

lipidic components which were found to resist the isolation and detection of plant bioactives. For purification, of these recovered CCEs, sequential liquid-liquid fractionation was further performed for the removal of intrinsic interfering moieties. After fractionation of crossponding CCEs of wild olive fruits, recovered with various extracting solvents, PRFs were obtained, and their yield was presented in Table 1. The yield of PRFs of tested fruits varied from 3.4 g/100g to 18 g/100g of CCE. The lowest yield (3.4%) of PRF was derived from CCE of aqueous methanol while the maximum yield (18%) of PRF was obtained from CCE of absolute ethanol.

According to the results of our study, aqueous ethanol was found to be better extracting solvent to recover higher yield of antioxidant compounds. The amount of extractable antioxidant molecules, extracted from the fruits of wild olive with different solvents, varied significantly ($p < 0.05$). These differences in the yield of extractable antioxidant components using various solvents was also described by Sultana et al., (2009). Hydroxymethanol and hydroxyethanol were very effective for the extraction of different phenolics from plant matrices (Chen et al., 2001; Jiao and Zuo, 2009; Manzoor et al., 2013; Shabir et al., 2011; Zuo et al., 2002). The results of present study advocates the effectiveness of hydroxymethanol for improved recovery of extractable phenolics and flavonoids. McDonald et al., (2001) and Sousa et al., (2011) reported the extraction yield (32 % by weight of original freeze-dried olive fruits), (19.38 %) from Australian freeze-dried olive fruit cultivars and Portuguese olive fruits cultivars using methanol which are found to be lower than of present study. Different factors including content of phenolic compounds, weather condition of site and nature of soil have been documented for the differences in the extraction yield (Mahmood et al., 2012).

3.2. Total phenolics (TP) and total flavonoids (TF) content

Over the last few decades, utilization of different parts of plants and plant-derived foods has extensively been increased due to the presence of phenolic bioactives in it. These phenolic and flavonoids are responsible for antioxidant activity of different fruits and vegetables (Katalinic et al., 2006). The TP and TF in CCEs/PRFs of tested fruits were determined as 0.79-2.95 GAE (g/100g) & 0.08-0.23 CE (g/100g), respectively (Table 1), where absolute acetone-derived CCE exhibited lowest content of TF (0.08 CE g/100g) and TP (0.79 g/100g of DW) while maximum amount of TF (0.23 CE g/100g) and TP (2.95 g/100g of DW) was found in CCE, extracted with hydroxyethanol.

The amount of TF and TP in PRFs of tested fruits varied over the range of 0.37-0.89 CE (g/100g) and 2.22-9.21 GAE (g/100g), respectively where PRF of absolute methanol-derived CCE contained the lowest content TF (0.37 CE g/100g) and TP (2.22 GAE) while maximum amount of TF (0.98 CE g/100g) and TP (9.21 GAE g/100g) was present in the PRF of hydroxyethanol-derived CCE.A significant difference ($p < 0.05$) was observed among the concentration of TF and TP in CCEs/PRFs extracted from the tested fruits with different solvents. Regardless of the lower extraction yield, the PRFs of tested fruits were found to contain higher concentration (3-4 times) of TF and TP than those of corresponding CCEs which showed phenolic components were successfully purified in PRFs.

There is no previous report available on assessment of TP and TF in the CCEs/PRFs, recovered from the wild olive fruits. The amount of TP in our present study was found to be higher than those of cultivated olive fruits (Arslan and Özcan, 2011; DeJong and Lanari, 2009; Ziogas et al., 2010), hydroxy methanol extract of raspberry fruit (Bobinaite et al., 2013), acidified-methanol extract of wild black berry (Radovanović et al., 2013) and absolute methanol extract of wild orange (Lala et al., 2020). The content of TF in wild olive fruits was found to be higher than those of (Nakilcioğlu and Hışıl, 2013), but in agreement with methanol extract of wild orange (Lala et al., 2020).

3.3. Reducing power of CCEs/PRFs

Reducing agents in CCEs/PRFs of wild olive fruits were used to reduce iron (III) into iron (II) to determine their reducing potential. The concentration of reducing agents in plant extract has a positive correlation with antioxidant activity of that plant (Joshi et al., 2010). Also, in this study, an increasing trend in the reducing power, which was enhanced linearly with the concentration of CCEs/PRFs (Table 2) was observed. he reducing potential of CCEs/PRFs, obtained from wild olive fruit was significantly ($p < 0.05$) varied from each other. Sousa et al., (2011) noticed similar trend of reducing power as a function of concentration of cultivated olive fruit extracts.

Table 1 Extraction yield, total phenolic and total flavonoid content in CCEs/PRFs of wild olive fruits.

Solvents	Yield (g/100 g of dry weight)		Total phenolics (GAE g/100 g of dry matter)		Total flavonoids (CE g/100 g of dry matter)	
	CCE	PRF	CCE	PRF	CCE	PRF
Absolute Methanol	34.93 ± 0.97^{d}	10.60 ± 1.28^{b}	1.52 ± 0.07^{c}	2.22 ± 0.16^{d}	0.09 ± 0.02^{d}	0.37 ± 0.04^{c}
Aqueous Methanol	45.73 ± 0.87^{b}	3.40 ± 0.45^{c}	2.79 ± 0.16^{a}	7.63 ± 0.57^{b}	0.18 ± 0.04^{b}	0.89 ± 0.06^{a}
Absolute Ethanol	37.45 ± 0.65^{c}	18.00 ± 0.93^{a}	1.81 ± 0.09^{b}	4.65 ± 0.49^{c}	0.14 ± 0.05^{c}	0.53 ± 0.04^{b}
Aqueous Ethanol	48.30 ± 1.06^{a}	3.90 ± 0.35^{c}	2.95 ± 0.18^{a}	9.21 ± 0.32^{a}	0.23 ± 0.04^{a}	0.98 ± 0.07^{a}
Absolute Acetone	23.10 ± 1.03^{f}	—	0.79 ± 0.11^{d}	—	0.08 ± 0.01^{d}	—
Aqueous Acetone	27.25 ± 1.43^{e}	—	1.74 ± 0.17^{bc}	—	0.12 ± 0.03^{c}	—

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p < 0.05$) of means among the extracting solvents.

3.4. DPPH free radical scavenging activity of CCEs/PRFs

DPPH is a stable nitrogen-centered free radical whose color was found to change from violet to yellow by attaining a proton from plant phenolics. The enhanced antioxidant capacity of different plant-derived extracts has been attributed to the increasing concentration of phenolic bioactives in it (Larrauri et al., 1999). CCEs/PRFs of tested wild fruits displayed substantial DPPH radical scavenging potential in terms of IC_{50} values as 0.03-0.78 mg/mL and 0.01-0.05 mg/mL, respectively (Table 2). Hydroxyethanol-derived CCE/PRF exhibited the maximum DPPH radical scavenging with lower IC_{50} values of 0.03 mg/mL and 0.01 mg/mL, respectively. Positive control (BHT) was found to found to express superior DPPH radical scavenging potential compared to other CCEs/PRFs. This antioxidant activity of tested CCEs/PRFs might be ascribed to the occurrence of antioxidant phenolics in them (Siddhuraju et al., 2002).

Antioxidant potential (in term of radical scavenging ability) of PRFs with IC_{50} (0.01-0.05 mg/mL) was higher ($p < 0.05$) in comparison to CCEs with IC_{50} (0.03-0.78 mg/mL) which might be due to increased phenolic antioxidants present in PRFs obtained by fractionation of CCEs. Though this is first report on evaluation of CCEs/PRFs of tested fruits for scavenging DPPH radical, however the DPPH radical scavenging potential of tested fruit's CCEs/PRFs was found to be comparable with that of olive cultivar's crude extract (Arslan, 2012), but higher than those of olive cultivars (Arslan and Özcan, 2011; Sousa et al., 2008), hydromethanolic extract of apple (Pires et al., 2018), hydroxyethanolic extract of wild apricot (Qin et al., 2019).

3.5. Antioxidant activity of CCEs/PRFs in linoleic acid system

Antioxidant activity of tested fruits' CCEs/PRFs was also determined by evaluating their potential to impede peroxidation in lipidic environment where peroxides were produced by the process of oxidation (Table 2). Peroxides in lipids have been reported to react with SCN^- to form a complex which can be quantified at 500 nm. The potential of various CCEs/PRFs of tested fruits to impede peroxidation in lipid molecules varied in the range of 41.83-78.65% and 82.12-89.31%, respectively where the maximum inhibiting potential was displayed by hydroxyethanol-derived CCE (78.65%) and PRF (89.31%) indicating its higher antioxidant capacity which is due to their higher content of antioxidant compounds compared to other extracts/fractions. However, absolute acetone-derived CCE (41.83%) and absolute methanol-derived PRF (82.12%) exhibited the lowest potential to retard the process of lipid peroxidation (Fig. 1).

According to the results of present study, PRFs of tested fruits were found to contain higher amount of phenolic antioxidant molecules which is a result of successful fractionation to remove impurities from recovered crude extracts, thereby their antioxidant activity in terms of inhibiting the process of peroxidation in lipids was higher in comparison to their corresponding CCEs. The synthetic antioxidant (BHT) expressed superior potential (92.01 %) for inhibiting lipid peroxidation compared to all CCEs/PRFs. This is first report on evaluation of CCEs/PRFs, recovered from wild olive fruits, to impede the peroxidation in lipids containing linoleic acid.

3.6. Antimicrobial attributes of CCEs/PRFs

Different plant material have been documented as a promising source of antimicrobial agents to treat various health disorders (Ahmad et al., 2011; Hussain et al., 2011). The evaluation of CCEs/PRFs, recovered from wild olive fruits, for antimicrobial potential against a panel of bacterial and fungal strains (Table 3) by measuring the inhibition zones and MIC (concentration of drug with lowest microbial growth) (Walsh et al., 2003).

The CCEs/PRFs, extracted from tested wild fruits, displayed highest bactericidal effected against *S. aureus* with greater inhibition zone (9-18 mm and 17-19mm) and MIC values (178-229 µg/mL and 151-171 µg/mL), respectively.

Table 2 Reducing power, DPPH radical scavenging activity, Inhibition of linoleic acid peroxidation of CCEs/PRFs recovered from wild olive fruits.

Solvents		Reducing Power (λ = 700 nm)				IC_{50} value (mg/mL)	Inhibition of linoleic acid peroxidation (%)
		5 mg	10 mg	15 mg	20 mg		
Absolute	CCE	0.54 ± 0.01	0.75 ± 0.04	0.99 ± 0.03	1.09 ± 0.05	0.16 ± 0.04^{c}	73.47 ± 3.69^{b}
Methanol	PRF	0.99 ± 0.08	1.05 ± 0.10	1.14 ± 0.06	1.20 ± 0.04	0.05 ± 0.02^{a}	82.12 ± 2.04^{b}
Aqueous	CCE	0.65 ± 0.02	0.88 ± 0.03	1.09 ± 0.05	1.14 ± 0.02	0.04 ± 0.02^{d}	76.26 ± 2.25^{a}
Methanol	PRF	1.15 ± 0.07	1.27 ± 0.06	1.30 ± 0.05	1.32 ± 0.08	0.02 ± 0.01^{b}	85.42 ± 2.78^{ab}
Absolute	CCE	0.04 ± 0.01	0.45 ± 0.02	0.71 ± 0.04	1.02 ± 0.04	0.09 ± 0.03 ^{cd}	72.67 ± 3.01^{b}
Ethanol	PRF	0.56 ± 0.05	0.69 ± 0.04	0.82 ± 0.09	1.08 ± 0.06	0.02 ± 0.01^{b}	84.88 ± 1.83^{ab}
Aqueous	CCE	0.51 ± 0.03	0.85 ± 0.02	1.11 ± 0.06	1.14 ± 0.05	0.03 ± 0.01^{d}	78.65 ± 2.69^{a}
Ethanol	PRF	1.01 ± 0.06	1.17 ± 0.04	1.21 ± 0.05	1.24 ± 0.11	0.01 ± 0.01^{b}	89.31 ± 2.91^{a}
Absolute	CCE	0.22 ± 0.01	0.35 ± 0.03	0.59 ± 0.05	0.76 ± 0.03	0.78 ± 0.07^{a}	41.83 ± 3.28^{c}
Acetone	PRF	–	–	–	–	–	–
Aqueous	CCE	0.36 ± 0.02	0.61 ± 0.02	0.96 ± 0.02	1.05 ± 0.02	0.49 ± 0.04^{b}	47.01 ± 2.86^{c}
Acetone	PRF	–	–	–	–	–	–

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p < 0.05$) of means among the extracting solvents.

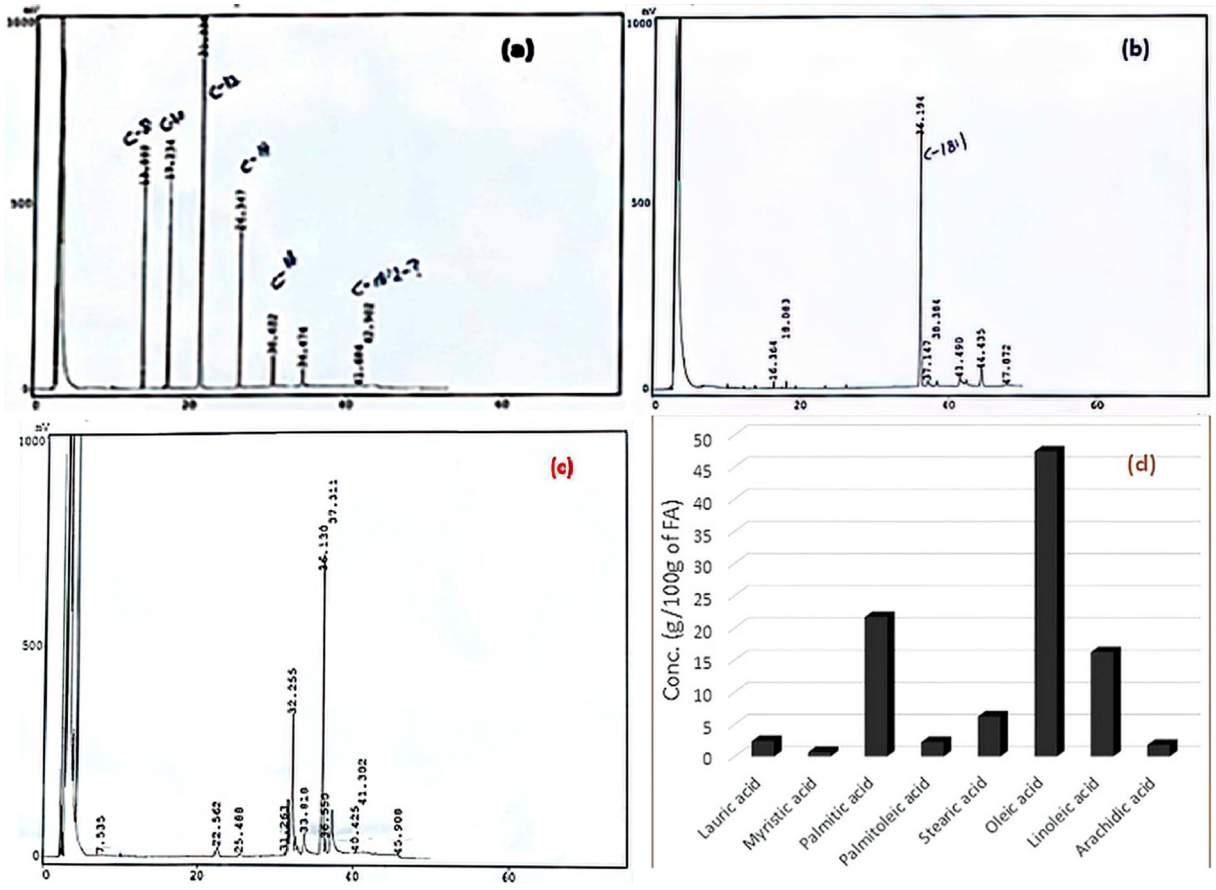

Fig. 1 Typical GC chromatograms: (a) saturated fatty acid standards, (b) unsaturated fatty acid standards, (c) wild olive oil FAMEs, (d) quantitation of fatty acids.

Hydroxyethanol derived CCE/PRF exhibited maximum antimicrobial potential against *S. aureus* with lowest MIC values 178 µg/mL and 151 µg/mL, respectively, while hydroxymethanol derived CCE/PRF displayed minimum antimicrobial potential against *B. cereus* with zone of inhibition (3-10 mm) and corresponding higher MIC values (241-290 µg/mL). The rifamycin (positive control) showed significantly ($p < 0.05$) higher antimicrobial attributes than those of tested CCEs/PRFs. According to the results of antimicrobial assay, CCEs/PRFs of tested wild fruits showed highest antifungal potential against *A. niger* with zone of inhibition (7-17 mm and 14-18mm) and corresponding MIC values (173-272 µg/mL and 164-210 µg/mL), respectively, while the lowest antifungal activity was recorded against *F. oxysporum* with zone of inhibition (2-9 mm; 6-10mm) and corresponding higher MIC values (246-340 µg/mL; 214-297 µg/mL), respectively (Table 4). The superior antibacterial and antifungal properties of CCE/PRF might be positively correlated with their enhanced concentration of phenolic antioxidant compounds.

The tested fruit's CCEs/PRFs exhibited higher bactericidal effect against *B. cereus*, *S. aureus* and *E. coli*. in comparison to: crude extract of wild olive against *E. coli* and *S. aureus* (Paudel et al., 2011); methanol-derived extract of wild orange against *staphylococcus sp*. (Lala et al., 2020); hydroxyethanolic extract of wild apricot against *B. cereus, E.coli* and *S. aureus* (Qin et al., 2019); acidified methanolic extract of wild black berry against *E.coli*. (Radovanović et al., 2013).

3.7. Biofilm inhibition potential of CCEs/PRFs

Various bacterial strains have been found to form biofilms for their survival against different types of stress including antibiotics. This predominant mode of growth with less vulnerability against different antimicrobial agents is a matter of concern. In order to control these infectious microbial strains, a variety of plant-derived antimicrobial agents have been isolated for their potential use in folk medicine systems (Prabu et al., 2006). The evaluation of CCEs/PRFs, recovered from tested fruits, was performed for their potential role in inhibiting the formation of bacterial biofilm (*S. aureus and E. coli*). According to the results of present study, CCEs/PRFs of wild olive fruits exhibited considerable potential to inhibit biofilm formation over the range of 51.53-75.68% and 69.16-81.25 %, respectively (Table 5). Among others, the lowest potential to inhibit the formation of biofilm was showed by acetone derived CCE (51.53-54.74 %) and PRF extracted from methanolic CCE (69.16-73.18 %), while aqueous-ethanolic derived CCE/PRF displayed maximum potential (72.56-75.68% and 80.07-81.25 %) to inhibit the formation of bacterial biofilm. This superior biofilm inhibition potential of hydroxylethanolic CCE/PRF is attributed to the occurrence of higher amount of plant bioactives in it. Due to the successful purification of phenolic components, PRFs were found to express superior ability to inhibit the formation of bacterial biofilms than those of crossponding CCE S. Rifamycin (87.91-89.43 %) being positive control was

Table 3 Antibacterial activity of CCEs/PRFs recovered from wild olive fruits.

Solvents	Zone of inhibition (mm)						MIC (μg/mL)					
	CCE			PRF			CCE			PRF		
	S. aureus	E.coli	B. cereus	S. aureus	E.coli	B. cereus	S. aureus	E.coli	B. cereus	S. aureus	E.coli	B. cereus
Absolute Methanol	13.0 ± 0.5^{d}	14.0 ± 0.3^{d}	6.0 ± 0.2^{d}	17.0 ± 1.1^{c}	16.0 ± 0.9^{cd}	7.0 ± 0.6^{cd}	215 ± 6^{b}	199 ± 7^{cd}	276 ± 8^{b}	171 ± 5^{a}	180 ± 8^{a}	225 ± 7^{ab}
Aqueous Methanol	17.0 ± 0.8^{bc}	16.0 ± 0.4^{c}	8.0 ± 0.3^{c}	19.0 ± 1.2^{b}	17.0 ± 0.7^{c}	8.0 ± 0.4^{c}	189 ± 8^{c}	194 ± 8^{cd}	251 ± 9^{c}	155 ± 8^{b}	163 ± 8^{b}	219 ± 8^{ab}
Absolute Ethanol	16.0 ± 0.8^{c}	15.0 ± 0.3^{cd}	4.0 ± 0.1^{e}	18.0 ± 1.0^{bc}	15.0 ± 1.5^{d}	6.0 ± 0.7^{d}	190 ± 6^{c}	201 ± 6^{c}	289 ± 8^{a}	169 ± 9^{a}	174 ± 7^{ab}	269 ± 10^{a}
Aqueous Ethanol	18.0 ± 0.6^{b}	19.0 ± 0.6^{b}	10.0 ± 0.4^{b}	19.0 ± 0.9^{b}	20.0 ± 1.2^{b}	11.0 ± 0.8^{b}	178 ± 6^{d}	167 ± 8^{d}	241 ± 7^{d}	151 ± 5^{b}	146 ± 6^{c}	209 ± 11^{b}
Absolute Acetone	9.0 ± 0.6^{e}	7.0 ± 0.2^{f}	3.0 ± 0.2^{e}	–	–	–	229 ± 7^{a}	240 ± 10^{a}	290 ± 12^{a}	–	–	–
Aqueous Acetone	12.0 ± 0.4^{cd}	11.0 ± 0.4^{e}	4.0 ± 0.2^{e}	–	–	–	213 ± 8^{b}	224 ± 8^{b}	281 ± 10^{ab}	–	–	–
Rifamycin	24^{a}	26^{a}	18^{a}	24^{a}	26^{a}	18^{a}	124^{e}	96^{e}	146^{e}	124^{c}	96^{d}	146^{c}

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p < 0.05$) of means among the extracting solvents.

Table 4 Antifungal activity of CCEs/PRFs recovered from wild olive fruits.

Solvents	Zone of inhibition (mm)						MIC(μg/mL)					
	CCE			PRF			CCE			PRF		
	A. niger	A. flavus	F. oxysporum	A. niger	A. flavus	F. oxysporum	A. niger	A. flavus	F. oxysporum	A. niger	A. flavus	F. oxysporum
Absolute Methanol	12.0 ± 0.7^{d}	10.0 ± 0.6^{d}	5.0 ± 0.2^{c}	14.0 ± 0.8^{c}	13.0 ± 0.4^{c}	6.0 ± 0.9^{c}	201 ± 9^{c}	235 ± 7^{c}	284 ± 7^{bc}	210 ± 9^{a}	218 ± 9^{a}	297 ± 7^{a}
Aqueous Methanol	17.0 ± 0.9^{b}	16.0 ± 0.7^{b}	9.0 ± 0.7^{b}	18.0 ± 0.5^{b}	17.0 ± 0.8^{b}	10.0 ± 0.7^{b}	173 ± 8^{d}	179 ± 8^{e}	246 ± 9^{d}	164 ± 7^{c}	176 ± 7^{c}	214 ± 7^{b}
Absolute Ethanol	15.0 ± 0.7^{c}	$12.0 \pm 0.7^{c}_{b}$	5.0 ± 0.4^{c}	15.0 ± 0.7^{c}	16.0 ± 0.6^{b}	7.0 ± 0.6^{c}	196 ± 9^{cd}	217 ± 8^{cd}	278 ± 10^{bc}	198 ± 8^{ab}	191 ± 6^{ab}	280 ± 10^{ab}
Aqueous Ethanol	16.0 ± 0.7^{bc}	15.0 ± 0.6^{b}	6.0 ± 0.3^{c}	17.0 ± 0.6^{b}	13.0 ± 0.3^{c}	7.0 ± 0.9^{c}	189 ± 8^{cd}	209 ± 10^{d}	269 ± 9^{c}	180 ± 7^{b}	203 ± 8^{b}	289 ± 9^{ab}
Absolute Acetone	7.0 ± 0.4^{f}	6.0 ± 0.3^{f}	2.0 ± 0.1^{d}	–	–	–	272 ± 10^{a}	295 ± 11^{a}	340 ± 11^{a}	–	–	–
Aqueous Acetone	10.0 ± 0.6^{e}	8.0 ± 0.3^{e}	5.0 ± 0.3^{c}	–	–	–	241 ± 8^{b}	259 ± 10^{b}	297 ± 10^{b}	–	–	–
Fluconazole	23^{a}	24^{a}	20^{a}	23^{a}	24^{a}	20^{a}	134^{e}	126^{f}	160^{e}	134^{d}	126^{d}	160^{c}

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p < 0.05$) of means among the extracting solvents.

found to express greater potential to inhibit the formation of bacterial biofilms in comparison to tested CCEs/PRFs of wild olive fruits. This is the first estimation of potential to inhibit biofilm formation by wild olive fruit's CCEs/PRFs.

3.8. Thrombolytic potential of CCEs/PRFs

The use of synthetic thrombolytic agents (urokinase and streptokinase) to treat thrombosis has severe health concerns (Collen, 1990) which prompts the need to explore different plant resources as an alternatives for their thrombolytic activity. A variety of plant-derived compounds including phenolic and flavonoid compounds have been reported to have thrombolytic potential (Maheshwari et al., 2011).

CCEs/PRFs of tested fruits exhibited thrombolytic activity in the range of 38.15-59.21 % and 56.24-71.42 %, respectively (Table 5), where minimum thrombolytic potential was displayed by absolute acetone's CCE (38.15 %) and absolute methanol-derived PRF (56.54 %), while hydroxyethanol-derived CCE (59.21%) and hydroxy methanol-derived PRF (71.42%) exhibited highest thrombolytic capacity which is due to the presence of enhanced content of phenolic compounds. According to the results of current assay, after the successful preconcentration of CCEs, PRFs were produced which exhibited superior thrombolytic attributes. Positive control (*streptokinase*) displayed higher (87.91-89.43 %) thrombolytic capacity in comparison to other CCEs/PRFs tested. There is no report available regarding the appraisal of tested fruit's CCEs/PRFs for thrombolytic potential in human, however, Dub & Dugani (2013) reported the thrombolytic effect of ethanolic leaves extract of olive (*Olea europaea L.*) in rabbits.

3.9. Haemolytic activity of CCEs/PRFs

Due to physiological and morphological attributes, erythrocytes have been a part of drug delivery systems in human body. Oxidative stress, induced by ROS and free radicals, damaged the membrane of erythrocyte to produce hemolysis (Ko et al., 1997). The evaluation of CCEs/PRFs, extracted from tested fruits, was performed for hemolytic potential which was found to vary over the range of 2.19-3.24%, 2.19-2.44%, respectively (Table 5). The maximum (3.24 % and 2.44%) and minimum (2.19 % and 2.19%) hemolytic activity was exhibited by CCEs and PRFs extracted with (absolute acetone, absolute ethanol), and (hydoxyethanol, hydroxyethanol), respectively.

The difference (1-2.50 times) of hemolytic potential between PRF (2.19-2.44 %) and CCEs (2.19-3.24 %) might be ascribed to presence of enhanced phenolic concentration in PRFs after successful fractionation of CCEs. PRFs of tested fruits rendered higher protection to erythrocytes to avoid hemolysis. Hemolytic potential of various CCEs/PRFs were found to vary due the efficacy of different solvents to recover phenolic antioxidant components from tested plant matrix. Positive control (PBS) exhibited lower hemolysis (1.54 %) than those of tested CCEs/PRFs of wild olive fruits.

3.10. Fatty acid composition of wild olive lipids

Lipidic content, total saturated and unsaturated fatty acids in tested wild olive fruit's oil was estimated to be 17.65 %,

Table 5 Biofilm inhibition, hemolytic and thrombolytic activity of CCEs/PRFs recovered from wild olive fruits.

Solvents	Biofilm inhibition (%)				Hemolytic activity (%)		Thrombolytic activity (%)	
	CCEs		PRFs		CCEs	PRFs	CCEs	PRFs
	S. aureus	E. coli	S. aureus	E. coli				
Absolute Methanol	65.43 ± 2.50^{e}	60.83 ± 1.90^{d}	73.18 ± 1.64^{e}	69.16 ± 1.71^{d}	2.57 ± 0.10^{b}	2.39 ± 0.04^{ab}	49.88 ± 1.75^{b}	56.54 ± 1.46^{b}
Aqueous Methanol	70.01 ± 3.35^{b}	67.15 ± 1.76^{c}	74.76 ± 1.57^{c}	75.31 ± 2.17^{c}	2.26 ± 0.12^{c}	2.19 ± 0.05^{b}	59.21 ± 1.09^{a}	71.42 ± 1.09^{a}
Absolute Ethanol	71.35 ± 2.25^{b}	71.57 ± 1.61^{bc}	77.09 ± 1.91 ^{cd}	69.40 ± 1.38^{d}	2.53 ± 0.08^{b}	2.44 ± 0.08^{a}	50.21 ± 1.47^{b}	57.42 ± 0.95^{b}
Aqueous Ethanol	72.56 ± 2.85^{b}	75.68 ± 1.73^{b}	81.25 ± 1.58^{b}	80.07 ± 1.87^{b}	2.19 ± 0.07^{c}	2.26 ± 0.04^{b}	57.47 ± 1.52^{a}	60.88 ± 2.38^{b}
Absolute Acetone	54.74 ± 2.28^{d}	51.53 ± 1.56^{e}	–	–	3.24 ± 0.09^{a}	–	38.15 ± 1.68^{e}	–
Aqueous Acetone	60.81 ± 1.56^{bc}	59.44 ± 1.89^{d}	–	–	2.50 ± 0.11^{b}	–	42.81 ± 0.95^{bc}	–
Control (Rifamycin)	87.91 ± 4.12^{a}	89.43 ± 3.94^{a}	87.91 ± 4.12^{a}	89.43 ± 3.94^{a}	–	–	–	–
PBS	–	–	–	–	1.54		–	
Triton X-100	–	–	–	–	100		–	
Water	–	–	–	–	–		3.84	
Streptokinase	–	–	–	–	–		92.34	

Values (mean ± SD) are average of three replicates, analyzed individually. Superscript letters in a same column display significant differences ($p < 0.05$) of means among the extracting solvents.

32.84 % and 65.92 %, respectively. The concentration of oleic acid (C18:1, 47.41 %) was found to be the highest as monounsaturated fatty acid, followed by the linoleic acid (C18:2, 16.24 %). Olive oil has been documented as one of the richest sources of oleic acid. High consumption of olive oil was found to control the problem of high blood pressure (Sousa et al., 2011; Tofalo et al., 2012; Uylaşer and Yildiz, 2013). Oleic acid (C18:1, 47.41 %) and palmitic acid (C16:0, 21.70 %) were determined to be as dominant mono-unsaturated and saturated fatty acid, respectively. Small concentration of lauric acid (2.40 %), strearic acid (6.22 %), myristic acid (0.70 %), palmitoleic acid (2.27 %) and arachidic acid (1.82 %) was also present in wild olive fruit's oil. Oleic acid and linoleic acid in wild olive oil was estimated to be higher compared to those of apple seeds (Pires et al., 2018), lower than those of olive cultivars reported by (Nergiz and Engez, 2000; Uylaşer and Yildiz, 2013). This difference in fatty acid content is associated with stage of fruit ripening, varieties, agro-climatic conditions and harvesting season.

3.11. Estimation of organic acids and sugars

Sugars and organic acids are major contributors in the taste development and organoleptic properties of different vegetables and fruits. Organic acids are obtained by formation and degradation of some components in plants. These organic acids are responsible for metabolic activity in plant matrices (Zhang et al., 2021). The evaluation of the tested fruits was conducted to detect different individual sugars including glucose, galactose, sucrose and xylose (Tables 6). The most dominant individual sugar was galactose (4.92 %) while small concentration of sucrose (2.75%), and glucose (0.73 %) were also identified in the fruits of wild olives. The results of present analysis advocate the use of wild olive fruit as source of natural sugars. Only succinic acid was detected in tested wild olive fruits at a concentration of 8.8 mg/100 g DW (Tables 6). Variation in the concentration of organic acids might be attributed to the different varieties of plants including vegetables and fruits, and agroclimatic condition of the selected site of experiment (Poyrazoğlu et al., 2002). Nergiz & Engez, (2000) and Patumi, et al., (1990) reported the detection of different sugars (fructose and glucose) and (glucose, fructose and mannitol) by analyzing olive fruit on HPLC coupled with refractive index detector (RID). Likewise, Marsilio et al., (2001) analyzed olive fruit extract for presence of sugars as trimethylsilyl (TMS) on GC/GCMS. Mannitol and glucose was determined as dominant individual sugars.

Arslan, (2012) investigated Turkish olive fruits for its physico-chemical properties and organic acid profiling. Tested fruit was found to contain total organic acids (3280–7088 mg/kg), tartaric, succinic, oxalic, malic, lactic, galacturonic and citric acids in the range of 84–353.2, 731.7–1038, 46.5–128.7, 37.8–372.8, 53.8–514.5, 228.7–453.0 and 1149.20–5148.80 m g/100 g fresh weight, respectively. Turkish olive fruit was analyzed on HPLC coupled with UV detector to estimate succinic, malic, citric and oxalic acids in the range of 389.00–1650.00, 764.50–4310.00, 417.02–46.37.50 and 6.6–345.00 mg/100 g, respectively (Nergiz & Engez, 2000). Cunha et al. (2001) reported the estimation of succinic, lactic and citric acids in Portuguese green table olive at a concentration of 0–25.8, 0–188.3 and 214.1–477.3 mg/100 g, respectively. Likewise, Günç Ergönül & Nergiz (2010) detected succinic, malic, citric and oxalic acids in olive fruit over the concentration of 518–614, 764.50–2056.50, 702.50–2030 and 66.60–75.70 mg/100 g, respectively.

3.12. Minerals profiling

Wild olive fruits were analyzed using ICP-OES for quantification of minerals present in it. Potassium, calcium, magnesium and sodium were detected at a concentration of 17955.22, 1095, 390 and 40 mg/kg as dominant minerals (Tables 7). The amount of potassium and sodium needs to be optimized to keep the blood pressure of the body in normal levels (NRC, 1989). The normal physiological functions of human body are performed by optimum intake of essential minerals

Table 7 Mineral profiling of wild olive fruits.

Elements	Concentration (mg/kg)	Elements	Concentration (mg/kg)
Al	150.95 ± 12.52	Se	5.52 ± 1.20
Ca	1095.73 ± 25.65	Cu	6.04 ± 0.09
Ba	2.04 ± 0.90	Cr	0.68 ± 0.02
Mg	390.69 ± 14.54	Na	240.19 ± 12.21
La	0.15 ± 0.02	Fe	111.70 ± 9.80
Mn	6.40 ± 0.05	B	25.43 ± 7.54
Ni	0.31 ± 0.02	Be	ND
Sr	5.23 ± 0.08	Co	0.21 ± 0.03
Zn	3.23 ± 0.06	P	154.66 ± 8.21
K	17955.22 ± 250.89	Sb	0.37 ± 0.03
Ti	25.19 ± 2.54	Si	47.61 ± 4.56
Cd	0.05 ± 0.02	V	ND
Pb	0.83 ± 0.02		

Values (mean ± SD) are average of three replicates, analyzed individually.
ND: Not Detected.

Table 6 Estimation of individual sugar (g/100 g DW) and organic acid (mg/100 g) in wild olive fruits.

Sugar content (g/100 g DW)					Organic acid (mg/100 g of dry matter)					
Glucose	Sucrose	Galactose	Xylose	Total Sugar	Succinic acid	Gluconic acid	Malic acid	Oxalic acid	Citric acid	Acetic acid
0.73 ± 0.03	2.75 ± 0.24	4.92 ± 0.76	ND	8.40	8.80 ± 0.25	ND	ND	ND	ND	ND

Values (mean ± SD) are average of three replicates, analyzed individually.
ND: not determined.

Table 8 Correlation analysis between TP and biological activities.

	TP	IC_{50} Value	Inhibition Potential	Antibacterial activity			Antifungal activity		Biofilm inhibition			Hemolytic activity	Thrombolytic activity
				S. aureus	E.coli	B. cereus	A. niger	A. flavus	F. oxysporum	S. aureus	E. coli		
TP	1												
IC_{50} Value	-0.780**	1											
Inhibition Potential	0.752**	-0.937**	1										
Antibacterial (S.aureus)	0.926**	-0.901**	0.893**	1									
Antibacterial (E.coli)	0.898**	-0.939**	0.929**	0.963**	1								
Antibacterial (B. cereus)	0.892**	-0.720**	0.762**	0.797**	0.859**	1							
Antifungal (A. niger)	0.897**	-0.922**	0.916**	0.982**	0.934**	0.759**	1						
Antifungal (A. flavus)	0.937**	-0.878**	0.885**	0.964**	0.919**	0.849**	0.978**	1					
Antifungal (F. oxysporum)	0.862**	-0.757**	0.708**	0.797**	0.737**	0.699**	0.859**	0.877**	1				
Biofilm inhibition (S. aureus)	0.828**	-0.890**	0.926**	0.966**	0.939**	0.703**	0.955**	0.899**	0.724**	1			
Biofilm inhibition (E.coli)	0.842**	-0.849**	0.842**	0.962**	0.940**	0.708**	0.909**	0.862**	0.620**	0.956**	1		
Hemolytic activity	-0.860**	0.869**	-0.707**	-0.826**	-0.867**	-0.742**	-0.806**	-0.798**	-0.794**	-0.731**	-0.762**	1	
Thrombolytic activity	0.919**	-0.899**	0.929**	0.945**	0.935**	0.879**	0.964**	0.985**	0.877**	0.900**	0.837**	-0.803**	1

Table 9 Correlation analysis between TF and biological activities.

	TF	IC_{50} Value	Inhibition Potential	Antibacterial activity			Antifungal activity			Biofilm inhibition		Hemolytic activity	Thrombolytic activity
				S. aureus	E.coli	B. cereus	A. niger	A. flavus	F. oxysporum	S. aureus	E.coli		
TF	1												
IC_{50} Value	-	1											
	0.572*												
Inhibition Potential	0.635**	-0.937**	1										
Antibacterial (S.aureus)	0.847**	-0.901**	0.893**	1									
Antibacterial (E.coli)	0.781**	-0.939**	0.929**	0.963**	1								
Antibacterial (B. cereus)	0.783**	-0.720**	0.762**	0.797**	0.859**	1							
Antifungal (A. niger)	0.781**	-0.922**	0.916**	0.982**	0.934**	0.759**	1						
Antifungal (A. flavus)	0.817**	-0.878**	0.885**	0.964**	0.919**	0.849**	0.978**	1					
Antifungal (F. oxysporum)	0.651**	-0.757**	0.708**	0.797**	0.737**	0.699**	0.859**	0.877**	1				
Biofilm inhibition (S. aureus)	0.795**	-0.890**	0.926**	0.966**	0.939**	0.703**	0.955**	0.899**	0.724**	1			
Biofilm inhibition (E.coli)	0.826**	-0.849**	0.842**	0.962**	0.940**	0.708**	0.909**	0.862**	0.620**	0.956**	1		
Hemolytic activity	-	0.869**	-0.707**	-	-	-0.742**	-	-0.798**	-0.794**	-0.731**	-	1	
	0.595**			0.826**	0.867**		0.806**				0.762**		
Thrombolytic activity	0.777**	-0.899**	0.929**	0.945**	0.935**	0.879**	0.964**	0.985**	0.877**	0.900**	0.837**	-0.803**	1

which are present in different fruits and vegetables. Moreno-Baquero et al., (2013) reported the estimation of sodium, potassium and calcium in seasoned cracked olives using atomic absorption spectrophotometer over the range of 10220–15466, 911–5567 and 980–3566 mg/kg, respectively as major minerals. Nergiz & Engez (2000) detected sodium, potassium, calcium, magnesium in the range of 11.1–32.8, 13951–16666, 23–56, 114–160 mg/kg as dominant minerals in Turkish olive fruits.

3.13. Correlation study among biological potential, TF and TP

Pearson method was employed to conduct correlation analysis at $P = 0.001$ of significance level (Table 8, 9) which showed TF and TP are positively correlated with inhibition potential, antimicrobial, biofilm and thrombolytic activities (P = 0.752, 0.862–0.937, 0.828–0.842 and 0.919), (P = 0.635, 0.651–0.847, 0.795–0.826 and 0.777) while IC_{50} value and haemolytic activity were found to have negative correlation (P = -0.780, -0.860), (P = -0.572, -0.595), respectively. We have already reported the presence of fourteen phenolic and flavonoid compounds in wild olive fruits (Ahmad et al., 2016), thus biological attributes of wild olive fruits are positive correlation with phenolic and flavonoid components in it.

The fruits of wild plants were explored for the occurrence of valuable nutrients and potential bioactive compounds due to the growing interest in their pharmacological benefits. Thus, the fruits of wild olives, native to Soon valley of Pakistan, were first time characterized for the presence of high-value nutrients and phenolic antioxidant compounds. The results of present research project have helped to bridge up the gap of scientific knowledge by authenticating the presence of phenolic antioxidants and high-value phytonutrients in the fruits of wild olive with notable biological activities which were rarely determined before. The results of present study advocate the potential utilization of the wild olive fruits in different formulation of nutra-pharmaceuticals and functional foods of multiple health benefits.

4. Conclusions

Wild fruits are promising source of phytonutrients and potent bioactives with multiple medicinal properties. Oleic and linoleic acids were detected as dominant unsaturated fatty acids in oil recovered from wild olive fruits. Appraisal of natural individual sugars (HPLC), organic acids (HPLC), valuable minerals (ICP-OES), total phenolics (TP) and total flavonoids (TF) and biological attributes of CCEs/PRFs, extracted from wild olive fruits, were performed. Potassium, calcium, magnesium, sodium and iron were identified as major minerals while succinic acid was detected as major organic acid in the fruits of wild olive. The aqueous-ethanolic extracts (CCEs/PRFs) furnished promising antioxidant and biological activities. There was a strong correlation among TF, TP and biological potential of CCEs/PRFs of wild olive fruits, indicating their biological activities were ascribed to the amount of total phenolic and flavonoids in the extracts. In view of bioactivities, the wild olive fruits could have potential application in nutra-pharmaceuticals industries.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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