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Research Article

BIOACTIVE COMPOUNDS AND ANTIRADICAL CAPACITIES OF DIVERSE EXTRACTS OF OLEA EUROPAEA L. LEAVES FROM PAKISTAN

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ABSTRACT:

Background: There has been an increase in interest in studies for the extraction of biologically active compounds from natural sources during the past decade. The Oleaeuropaea L. tree, occasionally referred to as the olive, is one of the members of the Oleaceae family and is very significant historically and economically, particularly in Mediterranean countries. Olive leaves have a lofty phenolic compound, which offer them antioxidant properties and a decreased risk of illness. **Aims:** The aim of this study was to determine the bioactive compounds (polyphenol, flavonoids) and antioxidant activities of the different olive leaves extracts. **Methods:** The percentage yield of various extract of olive leaves was measured and **t**he polyphenols was determined by Folin Ciocalteu reagent while the bioactive compounds flavonoids was estimated by using aluminum chloride method. The antioxidant activities of various extract of olive leaves were measured by DPPH and reducing power assay. **Results:** In this study the olive leaves extracts' maximum recovery yield (32.70%) of was found in methanol extracts and the total phenol concentrations varied from 3.64±0.11–135.70±8.2 (mg GAE/g) and flavonoids 1.20±0.07–26.35±1.20 (mg QE/g). Our results revealed that methanolic extract shown outstanding % Inhibition (DPPH) and reducing power activity. The capacity to lower the DPPH was in this order: methanol > water: methanol>water>chloroform > hexane extract at the same concentration. Similarly, methanolic extract, showed higher reducing power activity with absorbance which increases from 0.5701 to 1.0204 at concentration 0.1-0.5 mg/mL.
Conclusions: This concluded that the olive leaves were a potent source of bioactive compounds and its ability to scavenge free radicals and its reducing power is largely attributed to their phenolic compounds. However, more in-depth research is needed to identify the active compounds in these extracts that are responsible for these effects.

KEYWORDS:

Olive leaves, bioactive compounds, antioxidant capacities, DPPH, RPA

1 | INTRODUCTION

Olive is one of the most significant industrial crops in the world and is the famous woody oil species, that is native to the Mediterranean region. Its name derives from the Greek "elaia" and the Latin "olea" and it is a member of the Olea genus of the Oleaceae family. Its fruit and other items are consumed by people for their nutritional and health benefits. To meet the enormous demand for table olives and olive oil, which are olive products with health benefits, olive trees have been produced all over the world, particularly in South America, Pakistan, Australia, and

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China[1].During the seasons of pruning and harvest, olive leaves, a by-product of agriculture, are said to number more than 18 million tones per annum[2]. The olive tree's numerous components, most notably the leaves (Fig. 1), but also the fruits, oil, seed sand bark, have been used to treat a variety of ailments since ancient times.

Figure 1 Olive leaves

According to Hassen et al.[3] olive leaves extract is frequently used in phytotherapy to treat a variety of illnesses and is typically safe even at large dosages. Low-density lipoprotein cholesterol build-up in the artery walls, a significant cause of cardiac disease [4] can be prevented by using leaf extracts, which contain a variety of bioactive compounds, primarily oleuropein (Fig. 2) predominantly polyphenol that show affirmative effects on the parameters related to diabetes[5].

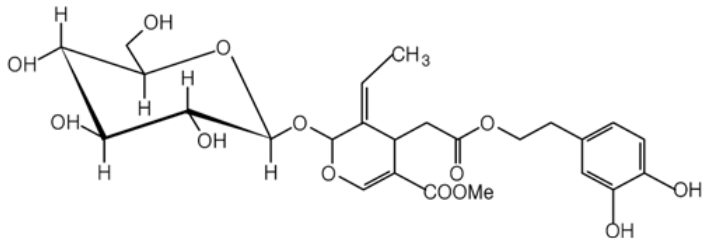

Figure 2 Structure of oleuropein Source: Yeon et al.,[6]

Along with oleuropein olive leaves also contains rutin, verbacoside, apigenin-7-glucoside, hydroxytyrosol, tyrosol, luteolin-7-glucoside and luteolin-7-rutinoside, as well as ligstrosideaglycone and decarboxymethyl ligstrosideaglycon [7] Recent research indicates that olive leaves extract has a positive impact on treating herpes simplex virus labialis[8] as well as treating the COVID-19.Recently, research on the fruit extract in experimental animals has shown that it improves chronic tiredness syndrome, hepatic lipid buildup, and antioxidant ability [9, 10].
Reactive oxygen species (ROS)that are harmful to biological systems are eliminated by antioxidants which function as a preventative against human illnesses. Two artificial antioxidants are butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) that have possess significant antioxidant activity, however the usage of synthetic antioxidants in general has generated debate due to reports of negative side effects[11]. As a result, there has been an increase in interest in substituting them with natural substances that have a variety of biological functions, such as inhibiting the assembly of ROS, scavenging free radicals, and altering the cell cycle. In addition, there is a lot of research being done today particularly on olive fruits, leaves, and oil, which has led to in vitro and in vivo evidence of antioxidant, anti-inflammatory, immune-modulating, antibacterial, antiviral, anti-cancer, anti-hypertension, anti-hyperglycemic, gastro protective, and several other biological functions[12.] The intention of this work was to assess the number of polyphenols, flavonoids as well as its antioxidants' ability of various extracts of olive leaves.

2 | MATERIAL AND METHODS

2.1 | PREPARATION OF SAMPLES

Olive leaves was obtained from olive garden Chakwal, Pakistan and identified by botanist from Department of Botany, GC University Lahore, Pakistan. For sample preparation 500g olive leaves were properly cleaned and rinsed with tap water. These leaves were first chopped into tiny pieces, dehydrated in a hot air oven at 50°C for 24 hours, then crushed into a fine powder by crushing in an electric grinder (Fig. 3) and kept in a dry, dark location for

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extraction [13].

Figure 3 Dry olive leaves and its powder

2.2 | EXTRACTION OF BOACTIVE COMPOUNDS

The procedure of Santas et al.,[14] was followed for the extraction process. For this, fine dried olive leaves powder (5g) was mixed to 50 mL of each of the solvents (hexane, chloroform, methanol, methanol: water (1:1) and water). These were shaken for 24 hours at room temperature at 150 rpm to homogenize then Whatman No. 1 filter was used to filter the extracts. To guarantee thorough extraction, the procedure was repeated two times. The extracts were then allowed to dry solidify at a temperature of 40 °C. The percentage yield was calculated by using the following equation.

$$\text{Percentage Yield} = \frac{\text{Weight of dry extract}}{\text{Weight taken for extraction}} \times 100$$

2.3 | ASSESSMENT OF POLYPHENOLS

The Folin-Ciocalteu technique Danial and Basudan [15], with some minor modifications Saeed[1] et al.,[16] was used to assess the polyphenols of olive leaves extracts in triplicate. In the tube, 0.1 mL (0.5 mg/mL) of each extract was added along with 0.5 mL of the Folin reagent (Merk). After 5 minutes 1.4 mL of 7.5% $Na_2CO_3$was added, and the mixture was allowed to react for 90 minutes at 25 °C. With the use of a spectrophotometer (UV-1700, Shimadzu Japan), the absorbance was determined at 760 nm. Three replica measurements of the samples were made. The findings were given as mg GAE/g of the extract sample.

2.4 | DETERMINATION OF FLAVONOIDS

With some minor modifications made by Saeed[2] et al.,[17] the colorimetric assay developed by Zhishen et al.,[18] was used to calculate the number of flavonoids in olive leaves extracts. 0.5 mL of the extract solution was combined with 0.5 mL of a 20 mg/mL $AlCl_3$ ethanol solution. After one hour of incubation at 25 °C, the absorbance was measured at 420 nm with a spectrophotometer (UV-1700, Shimadzu Japan) and the number of flavonoids was estimated that were represented as mg QE/g of the extract sample.

2.5 | DPPH FREE-RADICAL SCAVENGING ASSAY

With a little modification made by Saeed[3] et al.[19] the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical test was carried out spectrophotometrically as reported by Brand-Williams[20].In a test tube containing 2.9 mL of 0.004% methanol solution of DPPH, 0.1mL of each extract (0.1-0.5 mg/mL) was added. After 30 minutes of incubation at room temperature, the samples were evaluated for absorbance at 517 nmand the percentage of inhibition was calculated as follows.

$$\text{Inhibition } (\%) = (1 - A_S/A_C) \times 100$$

A_s is the absorbance of the test sample, whereas A_c is the absorbance of the control. The percentage of inhibition was calculated against extract concentration [21].

2.6 | REDUCING POWER ASSAY

The technique of Oyaizu, [22] was used to measure the reducing power of extracts of olive leaves with a slight modifications made by Saeed[4] et al., [23] Each extract's five concentrations (0.1-0.5 mg/mL) were mixedwith

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phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%) and 1.0 mL of distilled water. The mixture was incubated at 50 ºC for 20 minutes, and then (2.5 mL) of 10% trichloroacetic acid was added. The mixture was then centrifuged at 1000 g for 10 minutes (Centurian, Germany). The upper layer of the solution (2.5 mL) was then mixed with $FeCl_3$ (0.5 mL, 0.1%) and distilled water (2.5 mL). At 700 nm, a UV 1700 Shimadzu spectrophotometer was used to measure the absorbance. Higher absorbance of the reaction mixture indicated stronger reducing power.

2.7 | STATISTICAL ANALYSES

The results were analyzed using Analysis of Variance (ANOVA) and all experiments were carried out in triplicate. Duncan's Multiple Range Test at $P < 0.05$ and SAS software were used to identify significant differences between means.

3 | RESULTS AND DISCUSSION

3.1 | OBTAINABLE YIELDS

Fig. 4 displays the extract yield findings. The considerable variations in the availability of extractable components may account for the significant variability in olive leaves extraction yields in methanolic, water:methanolic, chloroform and hexane extracts. The methanolic extract (32.70%) produced a greater extraction yield following the water: methanolmixture. Similar outcomes were attained by Debib and Boukhatem, [24] who demonstrated that the olive leaf extract's maximal recovery 30% in methanol extract.

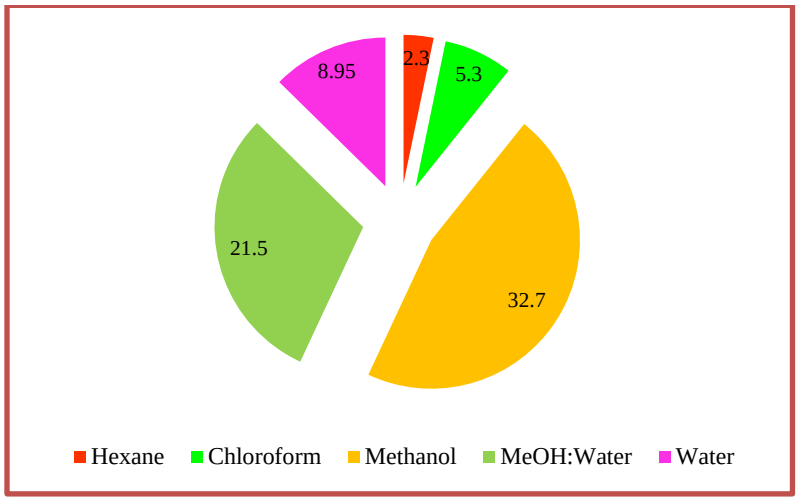

Figure 4 Percentage yield of extracts

3.2 | BIOACTIVE COMPOUNDS (POLYPHENOLS & FLAVONOIDS)

In the current investigation, the phenols and flavonoids of disparate olive leaves extracts were characterized that were shown in table 1. Hexane extract had the lowest phenols and flavonoids with 3.64±0.11 mg GAE/g and 1.20±0.07 mg QE/g, respectively. While methanol extract had the greatest levels of phenolics (135.7±8.2 mg GAE/g) and flavonoids (26.35±1.20 mg QE/g) followed by methanol: water, aqueous and chloroform extract. These findings are consistent with the given literature [25-28]. However, the quantity of total flavonoids compounds in our study was higher than Zhang et al.,[29] who narrated olive leaves range from (4.92–18.29 mg QE/g). Secondary metabolites found in plants called phenols are crucial for the spread of species and the development of disease resistance[30].

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Table 1 Bioactive compounds of diverse extracts of Oleaeuropaea leaves

Extracts	**Polyphenols** (mg GAE/g)	**Flavonoids** (mg QE/g)
Hexane	3.64 ± 0.11	1.20 ± 0.07
Chloroform	21.47 ± 0.95	7.10 ± 0.32
Methanol	135.70 ± 8.2	26.35 ± 1.20
MeOH+H_2O	93.50 ± 6.1	19.60 ± 1.15
Water	60.50± 1.23	9.25 ± 0.50

Data are represented as ± SD

3.3 | ACTIVITY TO SCAVENGE DPPH RADICALS

The ability of extracts to scavenge DPPH• was used to gauge their free radical scavenging abilities. Due to its simplicity and convenience, the DPPH radical is now often employed to evaluate the activity of radical scavengers[31]. Five different extract concentrations (0.1-0.5 mg/mL) were utilized to test the extracts' capacity to scavenge free radicals. According to their % Inhibition, all the extracts demonstrated their capacity to neutralize the DPPH radical (Fig. 5). All the extracts revealed their capacity to reduce the DPPH. However, the sequences of their reactivity were methanol extract > water: methanol > water > chloroform > hexane extract at same concentration. The findings of the cited literature [32-34] are consistent with this conclusion. Oleuropein an antioxidant found in olive leaf, was said to have anti-inflammatory qualities. According to Lins et al.[35] caffeic acid was also found in olive leaves which exhibit antioxidant action by scavenging superoxide anion. Contrarily, it has been demonstrated that the flavonoids, phenols and oleuropeosides found in olive leaves have significant antioxidant activity against free radicals. This is primarily due to the phenolic hydroxyl groups' redox properties and the structural connections between various chemical compounds[36].

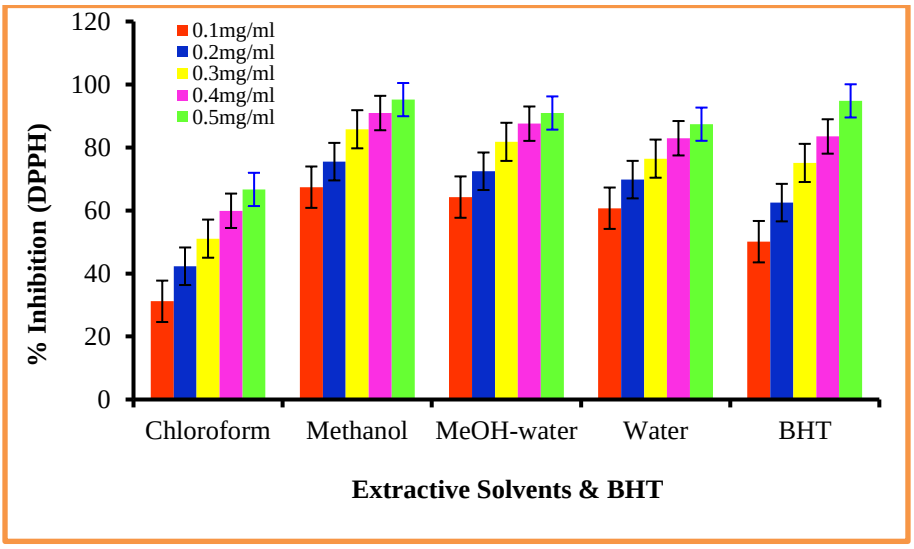

Figure 5 % Inhibition (DPPH) various extracts of olive leaves and BHT

3.4 | REDUCING POWER ACTIVITY

Antioxidants may be thought of as reductant chemicals that induce the Fe^{3+}/ferricyanide complex in the sample to become the ferrous (Fe^{2+}) form. As a result, the production of Perl's Prussian blue at 700 nm may be used to detect it. Wajiha and Qureshi[37]; Jomi et al.,[38]. Accordingly with an increase in absorbance, reducing power would also rise [39, 40]. The dropping clout of the different leaves extracts illustrated in Figure 6, with methanolic extract having the best results, increasing absorbance from 0.5701–1.0204 with a concentration increase from 0.1–0.5 mg/mL. This showed that most antioxidant capacity was found in methanol extract, followed by MeOH: water, and chloroform, while hexane extract had the lowest reducing power activity. These findings are in line with Altemimi et al.[41], who examined antioxidant characteristics in olive leaves and discovered that methanol extracts had the highest phenolic content and greatest reducing potential. A comparable finding was also obtained by Rafiee et al.,[42]; Nashwa et al.,[43];

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Lins et al.,[44] An important indicator of a substance's potential antioxidant action may be its reducing capability and higher absorbance of the reaction mixture implies more reductive potential[45].

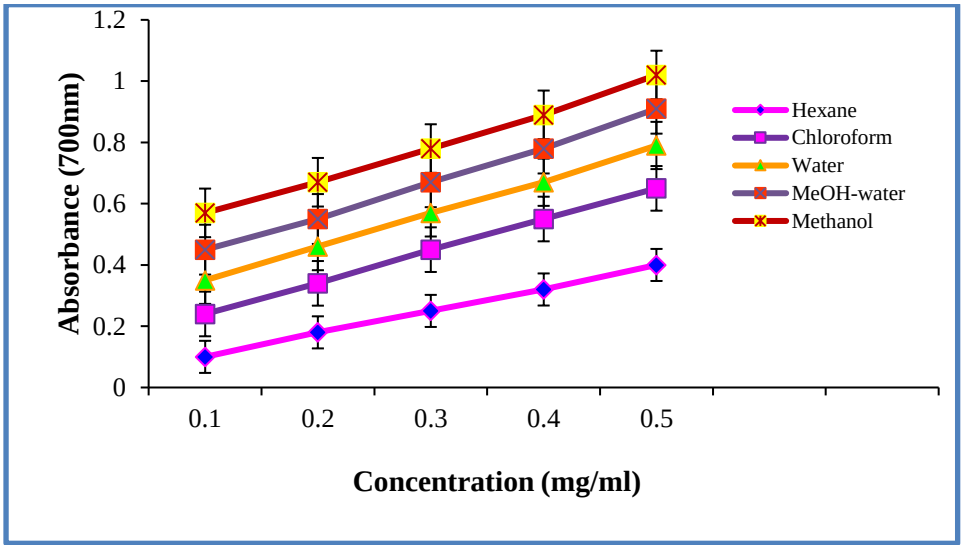

Figure 6 Reducing power activity of various extracts of olive leaves

4 | CONCLUSION

Olive leaves extracts are excellent sources of natural antioxidants including polyphenols and flavonoids and have been shown to have an antioxidant effect on reactive species with biological properties. However more research will be done to pinpoint substances that might clarify how antioxidants work. Further in vivo research is necessary to assess the antioxidant strength of olive leaves extracts.

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