DOI: https://doi.org/10.54393/df.v5i01.127

DIET FACTOR
Journal of Nutritional & Food Sciences
https://www.dietfactor.com.pk/index.php/df
ISSN (E): 2789-8105, (P): 2789-8091
Volume 5, Issue 1 (Jan-Mar 2024)

Original Article

Extraction and Characterization of Natural Antioxidants from Olive Leaves Powder

Muhammad Khalid Saeed[1*], Iram Shehzadi[2], Naseem Zahra[1], Shaheena Anjum[2], Zille Huma[2] and Khalil-Ur-Rehman[2]

[1]Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore, Pakistan
[2]Department of Chemistry, Riphah International University, Faisalabad, Pakistan

ARTICLE INFO

Keywords:
Olea europaea, Antioxidants, Polyphenols, Flavonoids

How to Cite:
Saeed, M. K., Shehzadi, I., Zahra, N., Anjum, S., Huma, Z., & Ur Rehman, K. (2024). Extraction and Characterization of Natural Antioxidants from Olive Leaves Powder : Extraction and Characterization of Natural Antioxidants . DIET FACTOR (Journal of Nutritional and Food Sciences), 5(01). https://doi.org/10.54393/df.v5i01.127

***Corresponding Author:**
Muhammad Khalid Saeed
Food and Biotechnology Research Centre,
PCSIR Laboratories Complex, Lahore, Pakistan
rosefbrc1@gmail.com

Received Date:19th February, 2024
Acceptance Date: 12th March, 2024
Published Date: 31st March, 2024

ABSTRACT

Olive (*Olea europaea* L.) leaves, which contain large amounts of phenols, are a common byproduct in the production of olive oil. When improperly processed, the by-products produced by the olive diligence can impairment the environment. Its leaves, which are made when olive trees are pruned and harvested and it is expected that foliage make up twenty five percent of pruning remains overall. These byproducts cost manufacturers money and have serious environmental implications. So, like other agronomical production wastes, partial reuse is something that should be pursued. These leftovers have a high concentration of beneficial chemicals, if isolated, may be used in food, cosmetics and nutraceutical sectors. **Objectives:** To extract the bioactive compounds polyphenols and flavonoids in 70% ethanol extract and its antioxidant activity was done. **Methods:** The polyphenols were quantified by Folin reagent and flavonoids by aluminum chloride methods respectively and the natural antioxidants were estimated by using free radical scavenging DPPH assay. **Results:** It was discovered that the 70% ethanol extract's total polyphenolic content was 65.50 ± 1.42 (mg GAE/g) and its total flavonoids were 11.85 ± 0.60 (mg QE/g). In 70% ethanol extract the % inhibition (DPPH) was 42.82±3.20-88.40±5.18 while BHT has the % inhibition (DPPH) 30.4 ± 2.50-80.50 ± 4.68 at concentration 0.1-0.5 mg/ml. **Conclusions:** The results indicating a noteworthy antioxidant activity in terms of radical scavenging activity. These results also wrapped up that the olive industry waste may be reutilized as a natural source of antioxidants in various sectors.

INTRODUCTION

Traditional medicine has utilized therapeutic plants since prehistoric times. Numerous chemical components have been shown to serve a variety of purposes, such as defense against herbivorous animals, fungus, insects and illnesses. Olive belongs to *Oleaceae* family and is significant historically, economically in all over the world especially in Mediterranean countries [1]. It is grown all throughout the globe, particularly in Pakistan, India, Australia and China, to meet the enormous demand for table olives and olive oil, both of which have health benefits [2]. Because of their extensive dispersion, they generate a large number of wastes that may be harmful to the environment. This majority of by-products are produced during olive tree trimming, fruit harvesting and oil production. The leaves were often burned or used as animal feed in the past that are squander resources and harm the environment while having little practical purpose [3]. Olive leaves have many health-promoting qualities since they contain a lot of biologically bioactive compounds such as phenolic acids, secoiridoids, pentacyclic triterpenes and flavonoids [4]. Recent study has demonstrated that its leaves have noteworthy bioactive substances, particularly TPC compounds, which encompass assorted actions/uses, including hypoglycemic, cardiovascular, improve brain

DOI: https://doi.org/10.54393/df.v5i01.127

function, anticancer, anti-nociceptive [5, 6]. The leaves of olive are a by-product of agriculture that build up over the pruning and harvesting seasons, with annual volumes predicted to exceed 18 million tones. In western nations, these natural leaves are promoted as an active component in supplementary alternative therapies and dietary supplements. Additionally, research and clinical trials show that olive leaves extract has a preventive impact on illness and due to polyphenols. As a result of the mounting evidence, numerous pharmacological and nutraceutical products as well as novel uses for olive leaves extracts have been developed [7]. Antioxidants function as a disease preventative by eradicating reactive oxygen species (ROS), which are damaging to biological systems. Due to allegations of harmful side effects, the usage of common synthetic antioxidants has become a contentious issue [8]. As a result, a lot of current research has focused on finding novel antioxidants that are similar to one another but come from natural sources.

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 construction of radicals and altering the cell cycle. Due to these facts and in order to make better use of these byproducts and waste, the current study was conducted.

METHODS

In April 2023, the leaves of olive were obtained from an olive garden in Chakwal, Pakistan and identified by a botanist. From Sigma-Aldrich, bought 2, 2-diphenyl-1-picrylhydrazyl, aluminum chloride, and the Folin reagent, methanol and ethanol. Distilled water was produced using a laboratory distillation machine. For the preparation of the olive leaves extract, one kilogram of olive leaves was first washed, dried for six hours at 50 to 60 ºC and then ground into a fine powder (Figure 1). 20 gram of dried olive leaves were extracted with 200 mL of 70 % ethanol, while being shaken at 180 rpm (Local PCSIR made shaker), kept in the dark at 25 ºC for 12 hours. The supernatant under reduced pressure (50 ºC) was evaporated and residue was collected and stored at 4 ºC for further research [9]. With some modifications to the procedure in literature [10] a colorimetric test was utilized to determine the amount of phenolic component in the extract by Folin method [11]. Briefly, 0.5 mL of Ciocalteu's phenol reagent was mixed with 0.1 mL of the sample. After three minutes, the combination received one milliliter of distilled water and 1.5 milliliter of saturated Na_2CO_3 solution. After the reaction was exposed to darkness for ninety minutes, the absorbance was measured at 765 nm. Total phenol content (TPC) values were computed by comparing the absorbance of each sample to a standard response curve created using gallic acid. A colorimetric test which was followed in order to measure the total flavonoid concentration in olive leaves 70% ethanol extract by given literature [12]. The results were represented as mg QE/g after a calibration curve was created using quercetin. In a nutshell, 2.5 mL of a solution of 2% $AlCl_3$, $_6H_2O$ was added to an aliquot of 0.5 mL of solution, stirred equally and after ten minutes absorbance at 367 nm was measured. To gauge the antioxidant activity the procedure was followed by [13] had described it, with a little tweaking from Saeed *et al.*, [14]. Briefly, 004% DPPH solution in methanol was made. 3 ml of this solution was mixed with 0.1–0.5 mg/mL extract in a test tube and after waiting for 30 minutes, absorbance was measured at 517 nm by using spectrophotometer. For each sample, a second reading was made. By using following formula, its antioxidant activity was measured:

$$\text{Scavenging Effect (\% I)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

The data were shown as ± standard deviation after three replications in every experiment of this study. SPSS Software version 21.0 was used for plotting graphs.

RESULTS

For extraction of polyphenols the dried olive leaves was and ground into a fine powder (Figure 1) and extracted in 70 % ethanol for maximum recovery. The results of polyphenolic quantity of 70% ethanol extract revealed a high phenolic content of 65.50 ± 1.42 (mg GAE/g) as shown in table 1.

(a) (b)

(c)

Figure 1: (a) Olive leaves with fruit, (b) Dried olive leaves, (c) Olive leaves powder

Flavonoids concentrations in this investigation were studied by using aluminum chloride and it was found that the concentration of flavonoids was 11.85 ± 0.60 (mg QE/g) as shown in table 1. Data are represented as mean ± SD.

DOI: https://doi.org/10.54393/df.v5i01.127

Table 1: TPC and TFC of 70% ethanol extract of olive leave powder

Bioactive Antioxidant Compounds	Values (mg/g)
Total Polyphenolic Content (mg GAE/g)	65.50 ± 1.42
Total Flavonoids Content (mg QE/g)	11.85 ± 0.60

The antioxidant activity of 70% ethanol was examined at various dilution (0.1–0.5 mg/mL) and percentage inhibition (DPPH) ranging from 42.82±3.20-88.40±5.18 (Figure 2). The antioxidant results were compared with standard synthetic antioxidant Butylated hydroxytoluene (BHT) which showed the % inhibition (DPPH) of 30.4 ± 2.50 - 80.50 ± 4.68 (Figure 3). The antioxidant activity of 70% ethanol of olive leaves was higher than standard antioxidant BHT.

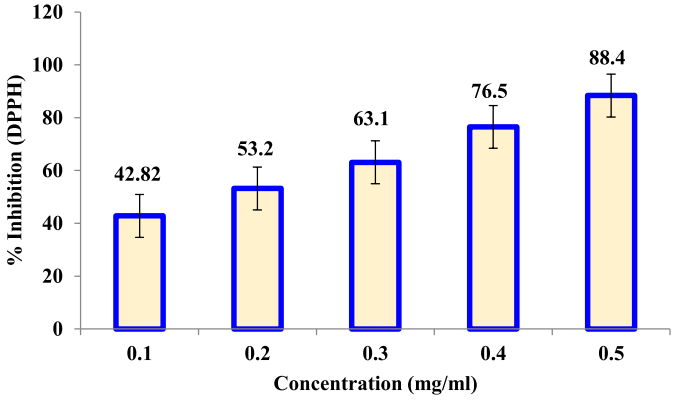

Figure 2: % Inhibition (DPPH) of olive leaves 70% Ethanol extract

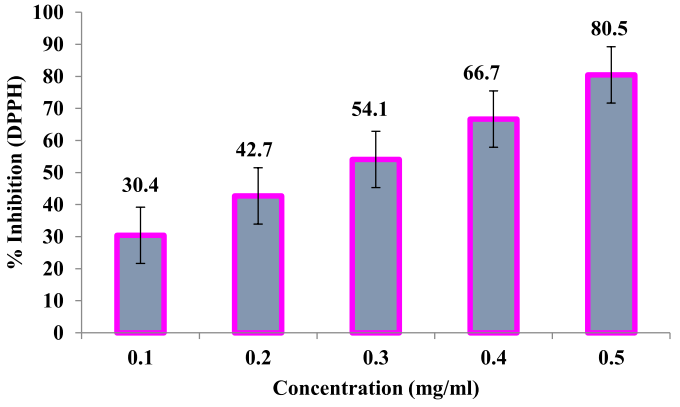

Figure 3: % Inhibition (DPPH) of standard antioxidant BHT

DISCUSSION

The results of this study were in accordance to those of a study by a researcher literature, who found that the phenolic content of olive alcoholic extract ranged from 21.3 to 22.6 mg GA/g [15]. The Aljeddani (2020) studied the ethanolic extract of olive leave by HPLCC and showed the numerous compounds in HPLC chromatogram [11] (Fig. 4). He also stated that the ethanolic extract had total phenolic content (21.47±0.05 mg GAE/g), while its water extract (10.5 ± 1.23 mg GAE/g) and these results are greater than this study. Numerous factors, including genetic make-up, the impact of the environment's climate, emergent period, warm temperatures, aridness, strong lunar contact, mud masterpiece and for extraction solvent employed can be utilized to explain this variation [16, 17]. The biological activities of plant extracts are due presence of its flavonoid content, narrated biological effects [18]. However, cultivar, growing environment and other variables can also affect flavonoid composition. This investigation's total flavonoid concentration was somewhat lower than that of research study, who found TFC of olive leave extracts with a highest value in methanolic extract (17.64 mg ± 0.07) and minimum value in petroleum ether (3.33 mg ± 0.07) [19]. Our findings also concur with given literature [20, 21].

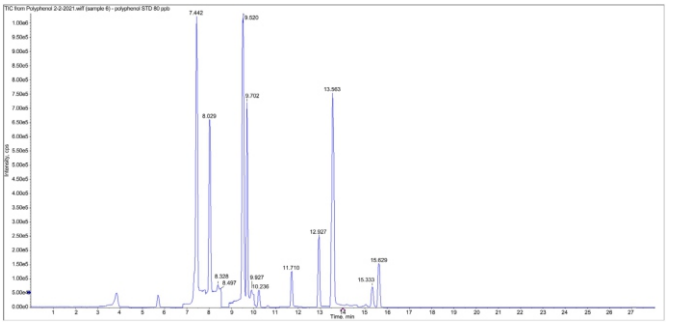

Figure 4: Chromatogram of ethanol extract of olive leaves numerous polyphenolic compounds detected: Base peak area obtained by HPLC ESI/MS-TOF [11]

Oxidative blemish is concerned in the etiopathogenesis of a lot of illness and antioxidants participates an imperative position in preventing the development and string of many diseases [22]. In this study DPPH radical was used to gauge their free radical scavenging abilities. As a result of its simplicity and convenience, DPPH radical is now frequently utilized in assessments of radical scavenging activity [23]. Previous investigations have confirmed the antioxidant activity of olive leaves of this study [24-26]. The DPPH test is based on the stable free radical with nitrogen-centered which have an ability to combine with H-donor compounds such phenolics and produce a stable molecule. According to the DPPH method, the antiradical potency of an antioxidant might be assessed by observing the decline in DPPH absorbance at 517 nm [27, 28]. These high antioxidant activities could be related to the presence of some bioactive compounds such as their high amount of total phenolic content (TPC) and flavonoids especially may be due to oleuropein, a well-known antioxidant derivative in olive leave extract [29, 30].

CONCLUSIONS

It is concluded that the 70% ethanol extract of olive leaves contains a considerable amount of TPC, TFC and free radical scavenging activity. Olive leaves can thus be regarded as a potential source of natural antioxidants.

DOI: https://doi.org/10.54393/df.v5i01.127

Authors Contribution

Conceptualization: MKS
Methodology: IS, ZH
Formal analysis: NZ, SA
Writing-review and editing: KUR, MKS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

The authors received no financial support for the research, authorship and/or publication of this article.

REFERENCES

[1] Zhang C, Xin X, Zhang J, Zhu S, Niu E, Zhou Z *et al.* Comparative evaluation of the phytochemical profiles and antioxidant potentials of olive leaves from 32 cultivars grown in China. Molecules. 2022 Feb; 27(4): 1292. doi: 10.3390/ molecules27041292.

[2] Steele EM, O'Connor LE, Juul F, Khandpur N, Baraldi LG, Monteiro CA *et al.* Identifying and estimating ultraprocessed food intake in the US NHANES according to the Nova classification system of food processing. The Journal of Nutrition. 2023 Jan; 153(1): 225-41. doi: 10.1016/j.tjnut.2022.09.001.

[3] Nunes MA, Pimentel FB, Costa AS, Alves RC, Oliveira MB. Olive by-products for functional and food applications: Challenging opportunities to face environmental constraints. Innovative Food Science & Emerging Technologies. 2016 Jun; 35: 139-48. doi: 10.1016/j.ifset.2016.04.016.

[4] Martínez-Navarro ME, Kaparakou EH, Kanakis CD, Cebrián-Tarancón C, Alonso GL, Salinas MR *et al.* Quantitative Determination of the Main Phenolic Compounds, Antioxidant Activity, and Toxicity of Aqueous Extracts of Olive Leaves of Greek and Spanish Genotypes. Horticulturae. 2023 Jan; 9(1): 55. doi: 10.3390/ horticulturae9010055.

[5] Orak HH, Karamać M, Amarowicz R, Orak A, Penkacik K. Genotype-related differences in the phenolic compound profile and antioxidant activity of extracts from olive (*Olea europaea* L.) leaves. Molecules. 2019 Mar; 24(6): 1130. doi: 10.3390/molec ules2 4061130.

[6] Popović M, Burčul F, Veršić Bratinčević M, Režić Mužinić N, Skroza D, Frleta Matas R *et al.* In the Beginning Was the Bud: Phytochemicals from Olive (*Olea europaea* L.) Vegetative Buds and Their Biological Properties. Metabolites. 2023 Feb; 13(2): 237. doi: 10.3390/metabo13020237.

[7] Lee SJ, Kim HS, Eom JS, Choi YY, Jo SU, Chu GM *et al.* Effects of Olive (*Olea europaea* L.) leaves with antioxidant and antimicrobial activities on in Vitro ruminal fermentation and methane emission. Animals. 2021 Jul; 11(7) :2008. doi: 10.3390/ani110720 08.

[8] Saeed MK, Zahra N, Saeed A, Shahzad K, Nawaz S, Abidi SH *et al.* Antimicrobial and antioxidant properties of water and methanolic extract of G. glabra native to Pakistan. Chemistry International. 2023 Apr; 9(2): 61-7. doi: 10.5281/zenodo.8117614.

[9] Jurišić Grubešić R, Nazlić M, Miletić T, Vuko E, Vuletić N, Ljubenkov I *et al.* Antioxidant capacity of free volatile compounds from *olea europaea* l. cv. oblica leaves depending on the vegetation stage. Antioxidants. 2021 Nov; 10(11): 1832. 10.3390/antiox10 111832.

[10] Saeed MK, Zahra N, Saeed A, Syed Q. Physicochemical Characteristics, Total Phenolic Content and Free Radical Scavenging Activity of Apple (Malus Domestica) Peel Powder: Physicochemical characteristics of Apple Peel powder. Pakistan BioMedical Journal. 2023 Feb: 07-11. doi : 10.54393/pbmj.v6i02.847.

[11] Aljeddani GS. Evaluation of phytochemical, antimicrobial, and antioxidant properties of wild versus cultivated olive leaves. Natural Science. 2022 Oct; 14(10): 448-61. doi: 10.4236/ns.2022.1410039.

[12] Moreno MI, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of ethnopharmacology. 2000 Jul; 71(1-2): 109-14. doi: 10.1016/ s0378-8741(99)00189-0.

[13] Sgherri C, Pinzino C, Navari-Izzo F, Izzo R, Quartacci MF. Antioxidant potential in lipophilic and hydrophilic extracts from medicinal herbs (Salvia officinalis and Echinacea angustifolia). a comparison between assays based on electron paramagnetic resonance and spectrophotometry. American Journal of Agricultural and Biological Sciences. 2012; 7(4): 417-24. doi: 10.3844/ajabssp.2012.417.424.

[14] Saeed MK, Zahra N, Abidi SH, Syed Q. Phytochemical screening and DPPH free radical scavenging activity of Aloe vera (Aloe barbadensis Miller) powder. International Journal of Food Science and Agriculture. 2022; 6(3): 301-8. doi: 10.26855/ijfsa.202 2.09.010.

[15] Rimawi WH, Salim H. Wild versus cultivated olive leaves extracts: Antioxidant activity, analysis of total phenolics and oleoropein content. 2016; 4: 61-86. doi: 10.15640/jcb.v4n2a6.

[16] Edziri H, Jaziri R, Chehab H, Verschaeve L, Flamini G, Boujnah D *et al.* A comparative study on chemical

DOI: https://doi.org/10.54393/df.v5i01.127

composition, antibiofilm and biological activities of leaves extracts of four Tunisian olive cultivars. Heliyon. 2019 May; 5(5). doi: 10.1016/j.heliyon.2019.e01604.

[17] Zaïri A, Nouir S, M'hamdi N, Bennani M, Bergaoui I, Mtiraoui A *et al.* Antioxidant, antimicrobial and the phenolic content of infusion, decoction and methanolic extracts of Thyme and Rosmarinus species. Current Pharmaceutical Biotechnology. 2018 Jun; 19(7): 590-9. doi: 10.2174/1389201019666180817141512.

[18] Cetinkaya H and Kulak M. Relationship between total phenolic, total flavonoid and oleuropein in different aged olive (*Olea europaea* l.) Cultivar leaves. African Journal of Traditional, Complementary and Alternative Medicines. 2016; 13(2): 81-5. doi: 10.4314/ajtcam.v13i2.10.

[19] Debib A and Boukhatem MN. Phenolic content, antioxidant and antimicrobial activities of "Chemlali" olive leaf (*Olea europaea* L.) extracts. International Journal of Pharmacology, Phytochemistry and Ethnomedicine. 2017; 6: 38-46. doi: 10.18052/www.scipress.com/IJPPE.6.38.

[20] Vogel P, Machado IK, Garavaglia J, Zani VT, de Souza D, Dal Bosco SM. Polyphenols benefits of olive leaf (*Olea europaea* L) to human health. Nutrición hospitalaria. 2015; 31(3): 1427-33. doi: 10.3305/ nh.2015.31.3.8400.

[21] Batçıoğlu K, Küçükbay F, Alagöz MA, Günal S, Yilmaztekin Y. Antioxidant and antithrombotic properties of fruit, leaf, and seed extracts of the Halhalı olive (*Olea europaea* L.) native to the Hatay region in Turkey. health. 2023; 1: 3. doi: 10.21603/2308-4057-2023-1-557.

[22] Liang W, He X, Bi J, Hu T, Sun Y. Role of reactive oxygen species in tumors based on the 'seed and soil'theory: A complex interaction. Oncology Reports. 2021 Sep; 46(3): 1-5. doi: 10.3892/or.2021.8159.

[23] Saeed MK, Zahra N, Saeed A, Abidi SH, Syed Q. *Syzygium Cumini* L. Seed A Potent Source of Fiber, Protein and Natural Antioxidants. Lahore Garrison University Journal of Life Sciences. 2022 Sep; 6(03): 252-67. doi: 10.54692/lgujls.2022.0603227.

[24] Khaliq A, Ahmad SD, Sabir SM, Khan A. Antioxidant activity and inhibitory effect of cultivars of Olive (Olea europaea) against lipid peroxidation in mice liver/Fare karaciğeri lipid peroksidayonuna karşı kültür zeytin'in Olive (Olea europaea) antioksidant ve inhibisyon etkileri. Turkish Journal of Biochemistry. 2015 Apr; 40(2): 188-96. doi: 10.5505/tjb.2015.03274.

[25] Lins PG, Pugine SM, Scatolini AM, de Melo MP. In vitro antioxidant activity of olive leaf extract (*Olea europaea* L.) and its protective effect on oxidative damage in human erythrocytes. Heliyon. 2018 Sep; 4(9). doi: 10.1016/j.heliyon.2018e00805.

[26] Palmeri R, Siracusa L, Carrubba M, Parafati L, Proetto I, Pesce F *et al.* Olive leaves, a promising byproduct of olive oil industry: Assessment of metabolic profiles and antioxidant capacity as a function of cultivar and seasonal change. Agronomy. 2022 Aug; 12(9): 2007. doi: 10.3390/agronomy12092007.

[27] Nazlić M, Kremer D, Grubešić RJ, Soldo B, Vuko E, Stabentheiner *et al.* Endemic Veronica saturejoides Vis. ssp. saturejoides–Chemical composition and antioxidant activity of free volatile compounds. Plants. 2020 Nov; 9(12): 1646. doi: 10.3390/plants9121646.

[28] Saeed MK, Zahra N, Saeed A, Shehzad K, Nawaz S, Abdi SH *et al.* Phytochemical Screening, Antioxidant and Antibacterial Studies of Various Extracts of Maranta arundinacea L. rhizomes. RADS Journal of Biological Research & Applied Sciences. 2023 Sep; 14(1): 11-7.

[29] da Silva AC, Paiva JP, Diniz RR, Dos Anjos VM, Silva AB, Pinto AV *et al.* Photoprotection assessment of olive (*Olea europaea* L.) leaves extract standardized to oleuropein: In vitro and in silico approach for improved sunscreens. Journal of Photochemistry and Photobiology B: Biology. 2019 Apr; 193: 162-71. doi: 10.1016/j.jphotobiol.2019.03.003.

[30] Cheurfa M, Abdallah HH, Allem R, Noui A, Picot-Allain CM, Mahomoodally F. Hypocholesterolaemic and antioxidant properties of *Olea europaea* L. leaves from Chlef province, Algeria using in vitro, in vivo and in silico approaches. Food and chemical toxicology. 2019 Jan 1;123:98-105. doi: 10.1016/j.fct.2018.10.002.