Potential aptitude of four olive cultivars as anticancer and antioxidant agents: oleuropein content

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Received: 22 November 2021 / Accepted: 31 January 2022 / Published online: 28 February 2022
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Abstract

Olea europaea L. (olive, Oleaceae) constitutes a source of many bioactive compounds, which have recognized benefits for both human health and technological purposes. The present article was carried out to evaluate the biological activity of oleuropein (an ester of 2-(3,4-dihydroxyphenyl) ethanol (hydroxytyrosol) which has the oleosidic skeleton that is common to the secoiridoid glucosides of Oleaceae). It occurred in leaf extracts of the four olive cultivars (Chemlali, Manzanilla, Picaul and Toffahi) as a source for some anticancer and antioxidant agents and their consequences on the action of Hordeum vulgare (barley). The total phenolic and flavonoid compounds were extracted from olive leaves by ethanol 95% then analyzed by high-performance liquid chromatography (HPLC). The study evaluates the anticancer activity of the ethanolic extract of olive leaves against breast and hepatocellular carcinoma cells showing high values. Also, the extract exhibited highly consequence on the antioxidant potentiality of barley which was assessed using the diphenyl picryl hydrazyl method (DDPH). These results pave the way for utilization of olive leaves as a source of natural anticancer and antioxidant agents.

Keywords
Olea europaea · Cultivars · Hordeum vulgare · Cancer · Antioxidants

1 Introduction

Olive (Olea europaea L.; Oleaceae) trees are considered as one of the oldest recognized cultivated plants in the world, as they have been planted for approximately 6000 years. The current cultivation covers about 9 million hectares worldwide (Espeso et al. 2021). It is one of the most globally important long-lived Mediterranean fruit trees and its habitat is determined by the Mediterranean climate (Kabbash et al. 2019). Acar-Tek and Ağagündüz (2020) and Sánchez-Gutiérrez et al. (2021) confirmed that traditional herbal medicines rely on the utilization of olive leaves to avoid and get relief from numerous illnesses, particularly in the Mediterranean countries. For example, leaves are utilized due to their antimicrobial (Pereira et al. 2007), antioxidant (Skrget et al. 2005), hypotensive (Khayyal et al. 2002), gastroprotective (Dekanski et al. 2009), hypoglycemic (Al-Azzawie and Alhamdani 2006), antiatherosclerotic (Wang et al. 2008), antiviral (Micol et al. 2005), antiarrhythmic (Somova et al. 2004), antitumor (Abaza et al. 2007), and antiinflammatory properties (Pieroni et al. 1996), and insulin sensitizing (González-Hedström et al. 2021) and antiproliferative activity (Goulas et al. 2009). The reported biological activities of the leaf extract have been related to specific phenolic compounds characteristic of the Olea genus. Oleuropein is the major component in olive fruits and leaves and accounts for most of the biological activities in the leaf extract (Hassen et al. 2015).

Nowadays, there is growing evidence that DNA damage caused by reactive oxygen species may be the first step in the progress of malignant cells can be repressed by consuming olive leaf extracts (Kontou et al. 2012). Menendez et al. (2008) estimated that oleuropein can destroy cancer cells depend on originating and storing energy from nutritional carbohydrates. Omar (2010) and Piroddi et al. (2016) confirmed that oleuropein can reach a concentration up to 140 mg g⁻¹ of dry matter in olive fruit and 60–90 mg g⁻¹

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(6–9%) of dry matter in olive leaves, while other reports indicate that oleuropein concentration in the leaves is as high as 19% (w/w).

Tori et al. (2019) and Echeverría et al. (2017) estimated that oleuropein as a single phenol has many natural actions, as it can be widely found on diverse kinds of cancer cells such as prostate, hepatocellular, pancreatic, colorectal and non-melanoma skin cancer. It also has subsidiary antioxidant potentiality to defend normal cells from oxidative injury.

Furthermore, El-Darier et al. (2016) and Kabbash et al. (2019) focused on the important role of olive leaves as a high antioxidant as well as having diverse healing properties. Additionally, the leaves that are wastes from olive oil manufacturing can be used as a vital product in the food industry.

The majority of research in this field has focused on the extraction and purification of oleuropein from leaves of different olive cultivars: Chemlali (CHM), Manzanilla (MAZ), Picaul (PIC) and Toffahi (TOF), to evaluate the antioxidant activity and therapeutic potential in cancer.

2 Materials and methods

2.1 Collection and preparation of plant material

Fresh and healthy leaves of four olive cultivars (Chemlali, Manzanilla, Picaul and Toffahi) were collected from Burg El-Arab and Dabaa regions (45 and 180 km west Alexandria, respectively). Leaves collected were dried in the shade for 15 days, thereafter ground in a Wiley Mill to a coarse uniform texture and then stored in glass jars until use.

2.2 Phytochemical screening

Qualitative test for flavonoids in plant materials was performed according to Harborne (1998). Extraction of total phenolic acid and total flavonoid assay were conducted using a modified method of Marinova et al. (2005). Thereafter, total phenolic and flavonoid contents were determined according to the method of Kim et al. (2003). Oleuropein was assayed by HPLC according to Boligon and Athayde (2014). Consequently, the content was calculated by comparing the oleuropein peak area in the test solution chromatogram and that in the standard solution chromatogram according to the following formula: \[ X = \frac{a^2 m^2 p^g}{a^1 m^1} \]

\[ a^1: \text{oleuropein peak area in the test solution chromatogram}, \ a^2: \text{oleuropein peak area in the standard solution chromatogram}, \ m^1: \text{mass of the test substance}, \ g, \ m^2: \text{mass of oleuropein in the standard solution}, \ p^g: \text{percentage of oleuropein in the oleuropein standard}. \]

2.3 Estimation of antiproliferative activity

2.3.1 Plant ethanolic extraction for cell line test (in vitro)

At the National Cancer Institute, Cairo, Egypt, according to the method adapted by Skelhan et al. (1990), the preparation of the ethanolic extract was carried out, by maceration of 100 g of plant powder in 95% denatured ethanol (1 L) for 24 h at room temperature. After that, the filtration of the solution was done, then the maceration process was done and all the procedures were repeated three times.

Two human tumor cell lines (Breast; MCF7 and liver; HEPG2 carcinoma cell lines) were used. Surviving fractions of cells throughout drug exposure were characterized graphically by IC50 values. Predominantly, a bar (P) parallel to the x-axis and intersecting the point 50% on the y-axis was constructed. In the next step, a bar was plotted parallel to the y-axis that started from the point of intersection of P with the dose–response plot. The IC50 could then be directly determined at the point of intersection with the x-axis.

2.4 Growth bioassay

2.4.1 Sterilization and germination of barley grains

For this purpose, barley grains were carefully selected with special characters with uniform size, shape and color. Then the process of grains’ sterilization was carried out by soaking them for 2 min in 2% CHLOREX and then washing many times with distilled water. The final step was soaking of the grains in well-aerated distilled water for 2 h.

2.4.2 Implementation

This experiment was accomplished for the verification of the consequence of varied concentration levels of olive leaves crude powder mixed (w/w) with sandy loam soils for the four the studied cultivars on the antioxidant activity of barley. To achieve this experiment, ten grains were sown in plastic pots (15 cm in diameter and 14 cm height) and thoroughly mixed (w/w) with 900 g soil under different concentrations (0.5, 1, 1.5 and 2%) of crude powder of the olive leaves. This test was done under standard research laboratory circumstances (24–25 °C day temperature, 19–21 °C night). One treatment was run as a control with 0% of crude powder. The pots were irrigated every 2 days with distilled water according to the amount calculated from the field capacity. After 21 days, harvesting of 5 standardized seedlings from each treatment was done. The seedlings were washed with...
tap water followed by distilled water to remove the adhering soil particles and finally swathed gently with filter paper.

2.4.3 Determination of antioxidant activity of barley

Harvested fresh samples (5 mg) were soaked in 5 ml 80% methanol overnight, then the extract was filtered by using Whatman No.4 filter paper and the filtrates were made up to 5 ml with 80% methanol. These extracts were ready for measurements of antioxidant activity.

Bandoniene et al. (2002) evaluated the antioxidant activity of phenol extracts through the utilization of the stable 2, 2-diphenyll–picrylhydrazyl radical (DPPH). The radical scavenging activities of the examined samples are expressed as percentage inhibition of DPPH and calculated according to the formula recommended by Molyneaux (2004):

$$A = \left( A_{\text{control}} - A_{\text{sample}} \right)/A_{\text{control}} \times 100,$$

where $A_{\text{control}}$ is the absorbance at 515 nm of the blank sample at time $t=0$ min and $A_{\text{sample}}$ is the final absorbance of the test sample at 515 nm.

2.5 Statistical analyses

All the data were subjected where appropriate to standard two-way analysis of variance (ANOVA). Qualitative data were described using number and percent (Kotz et al. 2006 and Kirkpatrick and Feeney, 2013). Also, correlation regression between quantitative variables was assessed by using a modern application polynomial curve fitting (Leung et al. 1992).

3 Results

3.1 Phytochemical screening

The phytochemical screening of leaf extract for the four olive cultivars is presented in Tables 1 and 2. Data showed that leaves were found to contain alkaloids, flavonoids, glycosides, phenols and oleuropein. In addition, data clearly demonstrated that the total flavonoids in Chemlali, Manzanilla, Picaul and Toffahi cultivars were 72.74 ± 2.00, 105.46 ± 3.00, 134.03 ± 2.45, and 156.05 ± 1.20 mg CE g⁻¹, respectively. For the total phenols, the values were 78.71 ± 1.92, 90.20 ± 2.67, 106.30 ± 2.06, and 153.05 ± 1.88 mg GAE g⁻¹, respectively. Similarly, oleuropein attained values of 35.02 ± 1.59, 47.24 ± 2.05, 55.42 ± 1.41, and 67.76 ± 0.64 mg/g dry weight by HPLC for the four cultivars, respectively. Additionally, Table 2 illustrates that the Toffahi cultivar attained the highest concentrations of all individuals of flavonoids and phenolic compounds except vanillic and ferulic acids, which attained their maxima in the Picaul cultivar.

3.2 Antiproliferative assay

Data represented in Fig. 1 demonstrates the dose–response curves produced by the SRB method fluctuated from 0 to 100 µg/ml. The law constructs that as the effect of the plant extract as an inhibitor is increased, the IC₅₀ values will be decreased. The powerful plant extracts can cause high inhibition at low concentration levels. Hence, the attained results showed that the MCF7 cell line is inhibited significantly in the Toffahi cultivar of olive with IC₅₀ = 14 µg/ml, while it is moderately suppressed with IC₅₀ = 15 and 16 µg/ml in the
Fig. 1 Effect of ethanolic leaf extracts of four olive cultivars (1. Chemlali, 2. Manzanilla, 3. Picaul and 4. Toffahi) on breast (MCF7) and liver (HEPG2) carcinoma cell lines.
Manzanilla and Chemlali cultivars, respectively. The Picaul cultivar obtained the lowest cell proliferation with MCF7 by IC50 = 25 µg/ml. In the instance of HEPG2 cell line, it is repressed significantly in the Manzanilla cultivar with IC50 = 12 µg/ml, while Chemlali obtained a moderate inhibition value with IC50 = 14 µg/ml, followed by the Picaul and Toffahi cultivars that achieved the same minimum inhibition values with IC50 = 15 µg/ml.

3.3 Antioxidant activity

Data recorded in Table 3 and Fig. 2 demonstrate that there is an increase in antioxidant activity of barley plant with the increase in the concentration level of olive leaf crude powder compared to control for cultivars CHM and MAZ. The values of antioxidant activity for the two cultivars were nearly the same and not significantly affected with the concentration levels. Concerning PIC and TOF, there were no apparent differences in the antioxidant values compared to the control. Nevertheless, the variation in antioxidant value was significant among the four cultivars.

4 Discussion

According to the statistics of the Economic Affairs Sector of the Egyptian Ministry of Agriculture, the cultivated area of olive trees in the year 2000 is 108.3 thousand feddan, while the cultivated area of the world is 9 million hectares (Mohamed and Saad El-Din 2002). Olive is an evergreen tree, well known for its edible fruits and oil. It is considered one of the most important crops related to the superior nutritional and medicinal value of its edible oil (Kabbash et al. 2019).

Tori et al. (2020) and El-Darier et al. (2021) estimated that there is a great demand in using and improving the traditional therapeutic approaches in cancer treatments and this is one of the most important remedial trials. The combination of these approaches with chemotherapy and radiotherapy is considered to be of a great benefit. Nowadays, there is a rising requirement for well-organized anticancer novel plans and techniques to improve the anticancer management and to avoid the developed drug confrontation as well as the side effects of cancer handling (Pucci et al. 2019). In this drive, the natural medicinal plants comprise massive quantities of innovative ingredients that can be used successfully in the up-to-date pharmaceutical investigations. Now, more than 60% of the antitumorigenic drugs are derivatives from natural sources. So, the usage of natural substitute medications has become immediate and essential (Talib et al. 2020).

Kwan et al. (2017) accepted that the rate of cancer can be suppressed by intake of the Mediterranean food, so olive products are widely used relying on their history in traditional medicine and phytochemical studies. These studies have recognized some constituents extracted from the products of olive tree that have many cytotoxic consequences. Bisignano et al. (1999) and Isidori et al. (2005) reported that oleuropein (High molecular weight polyphenols) plays a crucial role in the suppression of microbial growth as it attained a high toxic activity.

The existing study demonstrates that leaf extracts of four different olive cultivars inhibited efficiently the cell proliferation of MCF7 and HEPG2. For instance, breast cancer cell line (MCF7) exhibited the highest inhibition value in the Toffahi cultivar (associated with high oleuropein content)

| Table 3 | Variation in antioxidant activity ± SD of barley (21 days after sowing) as affected by different concentrations (%) of four olive cultivars (Chemlali, Manzanilla, Picaul, and Toffahi) leaf crude powder |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Concentration (%) | Chemlali (n = 3) | Manzanilla (n = 3) | Picaul (n = 3) | Toffahi (n = 3) | F1 | p1 |
| Control | 18.9±0.10 | 18.9±0.0 | 18.9±0.1 | 18.9±0.1 | 0.0 | 1.000 |
| 0.5 | 36.6±0.20 | 34.0±0.5 | 17.6±0.1 | 17.3±0.3 | 3303.051* | <0.001* |
| 1.0 | 37.6±0.1 | 36.5±0.5 | 16.6±0.2 | 17.6±0.1 | 5149.774* | <0.001* |
| 1.5 | 39.0±0.2 | 33.6±0.1 | 19.2±0.2 | 17.8±0.1 | 13,296.0* | <0.001* |
| 2.0 | 36.1±1.1 | 33.0±0.5 | 16.8±0.4 | 16.5±0.5 | 695.743* | <0.001* |
| F2 | 791.485* | 968.191* | 81.462* | 30.689* |
| p2 | <0.001* | <0.001* | <0.001* | <0.001* |

Data were expressed by using mean ± SD

F1, p1: F and p values for ANOVA test for comparing between the four groups

F2, p2: F and p values for ANOVA test for comparing between different concentrations

Significance between groups was done using post hoc test (Tukey)

Means with common letters in the same row are not significant

Means with different letters in the same row are significant

*Statistically significant at p ≤ 0.05
and moderate suppression in Manzanilla as well as Chemlali cultivars. Nevertheless, MCF7 revealed the lowest inhibition value with Picaul.

Sirianni et al. (2010) showed that oleuropein has an effect in the suppression of cell proliferation of MCF-7 cells, as well as it can enhance the level of ROS and induces apoptosis in MCF-7 cell lines. Moreover, Elamin et al. (2013) conducted an experiment in vivo to inhibit breast cancer through applying a mixture of doxorubicin and oleuropein that resulted in activating apoptosis, while the combination treatment of doxorubicin and oleuropein (48.7 mm³) had greater effectiveness than either doxorubicin (69 mm³) or oleuropein (79 mm³) alone. Shamshoum and Vlavcheski (2017) explained the precise steps by which oleuropein disturbs the tumor cells through bringing into the target cells specific transporters or binding to specific receptors. In clarification, Han et al. (2009) reported that by exposing the cells of MCF-7 human breast cancer to oleuropein, there was a great reduction in the cell feasibility, high inhibition in cell proliferation, excessive induction in apoptosis and all these consequences were accompanied by improvement in the cell number. Additionally, El-Darier et al. (2018) posulated that both thyme and clove oils have cytotoxic effect on breast cancer cell lines (MCF7); results showed that the increase in the concentrations of both oil types stimulates the inhibition of growth of MCF7 cell lines. Also, El-Darier and Abdelhady (2017) determined the anticancer activity of the fruit pulp of Annona squamosa ethanolic extract against several cancer cell lines such as lung (H1299 & A549), liver (HEPG2), prostate (PC3), cervical (HELA), larynx (HEP2), breast (MCF7) and intestine (CACO).

Liver cancer cell line (HEPG2) in the present study showed high suppression of cell proliferation with Manzanilla and Chemlali cultivars and achieved moderate inhibition with Picaul and Toffahi cultivars. Yan et al. (2015) investigated that oleuropein can decline significantly the cell
grapeseed extract (Durlu and Ozkaya, 2011). Antioxidant capacity of oleuropein is 400% higher than vitamin C and twice that of green tea or vanillic acid. Antioxidant capacity of oleuropein is different compounds are also present in olive leaves, e.g., caffeic acid, rutin, hesperetin, quercetin, gallic acid, catechol and vanillic acid. Antioxidant capacity of oleuropein is 400% higher than vitamin C and twice that of green tea or grapeseed extract (Durlu and Ozkaya, 2011).

In the present study, oleuropein in the olive leaf extract attained values varying from 35 to 67 mg/g dry weight depending upon the cultivar. In addition to oleuropein, different compounds are also present in olive leaves, e.g., caffeic acid, rutin, hesperetin, quercetin, gallic acid, catechol and vanillic acid. Antioxidant capacity of oleuropein is 400% higher than vitamin C and twice that of green tea or grapeseed extract (Durlu and Ozkaya, 2011).

In the present study, cultivars CHM, MAZ, PIC and TOF attained oleuropein values of 35.02 ± 1.59, 47.24 ± 2.05, 55.42 ± 1.41 and 67.76 ± 0.64 mg/g dry weight, respectively. The variation was highly associated with the level of antioxidant activity of germinated barley grains. The antioxidant levels in barley seedlings were highly maximized when treated with olive crude powder in the first two cultivars (CHM and MAZ) relative to the control. Contrarily, the two last cultivars (PIC and TOF) had no effects on the antioxidant activities relative to the control. Yang et al. (2021) investigated the effects of different drying methods on the antioxidant activity and antioxidants in sweet potato (Ipomoea batatas L. Lam.) tubers. The dried sweet potatoes in the microwave possessed the highest antioxidant activity, while the lowest activity was observed in hot air-dried samples. Similarly, Kovacik et al. (2014) postulated that the antioxidant activity of barley that is exposed to N deficiency is highly affected, as it decreased in the shoots but increased or not affected in the roots.

Boantă et al. (2019) estimated that barley is a unique, old and cultivated plant that plays a significant role in the progress of human civilization, agronomic, physiological, genetic sciences and plant breeding. From ancient eras, it was added to the food of both humans and animals. Additionally, barley achieved much therapeutic potentialities that were revealed over time. Barley can be administrated in many forms such as juices or powder made from the young leaves of the plant, barley water obtained from barley grains and the green barley that is very valued for the human body as it has a brilliant healing effect. Moreover, Sharma et al. (2021) proved that barely has numerous beneficial properties; for example, the shoots are diuretic, while the seed are emollient, demulcent, expectorant, digestive and nutritive. It can be consumed internally as a nutritious food or as barley water and has exceptional usage for babies, as it can decrease excessive lactation. It can be also administrated as a poultice for burns and wounds. Recent research has shown that barley may be of aid in the treatment of hepatitis, while it may help to control diabetes. Also, barley can be used for lowering the blood cholesterol levels and preventing bowel cancer.

Omwamba et al. (2013) showed that the juice of barley seedlings comprises several vital constituents such as vitamins B1, B2, B6, B12, beta carotene and folic acid. Also, it contains the most needed minerals such as phosphorus, potassium, calcium, iron and magnesium. Furthermore, other ingredients exist such as chlorophyll, amino acids, protein, fiber, and enzymes. Vegetarians can evade vitamin B12 deficiency by addition of dehydrated barley seedling juice to their diets. Also, the antioxidant activity of barely grain extract was assessed by several methods; in vitro and in vivo. All the outcomes exhibited the high antioxidant activities which demonstrated by its aptitude to chelate ferrous ions and scavange hydroxyl and superoxide radicals.

Gromkowska-Kepka et al. (2021) assessed that barley is a highly useful plant that can be utilized as nutricosmetic because of the many biologically dynamic compounds. Preparations of young barley contain high total polyphenolic content and promising total antioxidant potential. There are several antioxidant elements such as zinc, copper, and selenium. Also, the examined products are safe with respect to toxic elements such as lead, cadmium and mercury, as well as absence of cytotoxic consequence on the normal cells.

The present study can direct our awareness to the principal aim of barley cultivations and conclude that if barley is planted to be used in herbal medicine as antioxidant, it is preferred to be cultivated under CHM and MAZ olive trees. This is therefore an essential conclusion to further elucidate the potential health effects of phytochemical antioxidants in diet. El-Kenany et al. (2017) elucidated that the influence of 1–3% concentration levels of Nicotiana glauca shoot crude powder on the total proteins and antioxidants of Triticum aestivum was positively remarkable.

5 Conclusion

1. Some olive cultivars may be used as a source of oleuropein which may act with the current traditional chemotherapy treatments of breast (Toffahi) and liver (Manzillla) cancer.
2. Toffahi olive cultivar attained the maximum oleuropein content.
3. Application of CHM and MAZ olive cultivar crude powder in barley cultivations enhances the production of antioxidants, a biomarker for medicinal value of barley.
Funding Open access funding provided by The Science, Technology & Innovation Funding Authority (STDF) in cooperation with The Egyptian Knowledge Bank (EKB).

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References

Abaza L, Talorete TP, Yamada P, Kurita Y, Zarrouk M, Isoda H (2007) Induction of growth inhibition and differentiation of human leukemia HL-60 cells by a Tunisian germou olive leaf extract. Biosci Biotechnol Biochem 71(5):1306–1312

Acar-Tek N, Ağagündüz D (2020) Olive Leaf (Olea europaea L. folium); potential effects on glycemia and lipidemia. Ann Nutr Metab 76(16):10–15

Al-Azzawie H, Alhamdani MSS (2016) Hypoglycemic and antioxidant effect of oleuropein in allaxon-diabetic rabbits. Life Sci 78(12):1371–1377

Bandioniene D, Murkovic M, Tadic V, Markovic G, Arsic M, Mielcarek K, Soroczynska J, Socha K (2021) Chemical composition and protective effect of young barley (Hordeum vulgare L.) dietary supplements extracts on UV-treated human skin fibroblasts in vitro studies. Antioxidants 10(1402):1–30

Han J, Talorete TPN, Yamada P, Isoda H (2009) Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. Cytotechnology 59(2013):310–316

El-Darier SM, Ahmed HA, Abdel Razik MS, Alłam ES (2016) Probable organic fertilizer production from olive mill solid waste. Asian J Appl Sci 4(4):979–1003

El-Darier SM, El-Ahwany AMD, Elkeneny ET, Abdeldaim AA (2018) An in vitro study on antimicrobial and anticancer potentiality of thyme and clove oils. Rend Fis Acc Lincei J 29:131–139

González-Hedström D, García-Villalón AL, Amor S, Fuente-Fernández M, Almodóvar P, Prodanov M, Priego T, Martín AI, Inarejos-García AM, Granado M (2021) Olive leaf extract supplementation improves the vascular and metabolic alterations associated with aging in Wistar rats. Sci Rep 11(8188):1–16

Hassen I, Casabianca H, Hosni K (2015) Biological activities of the natural antioxidant oleuropein: Exceeding the expectation- a mini-review. J Funct Foods 18:926–940

Hassan I, Talorete TP, Yamada P, Isoda H (2009) Anti-proliferative activity of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. Cytotechnology 59(2013):45–53

Harborne JB (1998) Phytochemical methods, a guideto modern techniques of plant analysis, 3rd edn. UK, London, p 317

El-Darier SM, Ahmed HA, Abdul Razik MS, Alłam ES (2016) Probable organic fertilizer production from olive mill solid waste. Asian J Appl Sci 4(4):979–1003

El-Darier SM, El-Ahwany AMD, Elkeneny ET, Abdeldaim AA (2018) An in vitro study on antimicrobial and anticancer potentiality of thyme and clove oils. Rend Fis Acc Lincei J 29:131–139

El-Darier SM, Kamal SA, Marzouk RI, Nour IH (2021) Anti-proliferative activity of Launaea fragilis (Asso) pau and Launaea nudicaulis (L.) hook f. extracts, J Sci Tech Res 35(2):27492–27496

El-Kenany ET, El-Darier SM, Abdellatif AA, Shakhl SM (2017) Allelopathic potential of invasive species; Nicotiana glauca Graham on some ecological and physiological aspects of Medicago sativa L. and Trifolium aestivum L. Rend Fis Acc Lincei J 28:159–167

Espeso J, Isaza A, Lee JA, Sörensen PM, Jurado P, Bustillos RJ, Olaizola M, Arboleyca JC (2021) Olive leaf waste management. Front Sustain Food Syst 5(660582):1–13

González-Hedström D, García-Villalón AL, Amor S, Fuente-Fernández M, Almodóvar P, Prodanov M, Priego T, Martín AI, Inarejos-García AM, Granado M (2021) Olive leaf extract supplementation improves the vascular and metabolic alterations associated with aging in Wistar rats. Sci Rep 11(8188):1–16

Goulas V, Exarchou V, Trogiannis A, Psomiadou E, Fotis T, Brinasoulis E (2009) Photochemicals in olive-leaf extracts and their anti-proliferative activity against cancer and endothelial cells. Mol Nutr Food Res 53(5):600–608

Guzman-Morales C, Arevalo-Pabon A, Arboleya JC (2021) Olive leaf extract supplementation improves the vascular and metabolic alterations associated with aging in Wistar rats. Sci Rep 11(8188):1–16

Hassen I, Casabianca H, Hosni K (2015) Biological activities of the natural antioxidant oleuropein: Exceeding the expectation- a mini-review. J Funct Foods 18:926–940

Isidori M, Lovorgina M, Nardelli A, Parrella A (2005) Model study on the effect of 15 phenolic olive mill wastewater constituents on seed germination and Vitis vinifera metabolism. J Agric Food Chem 53(21):8414–8417

Kabbash EM, Ayoub MB, Abdel-Shakoura ZT, El-Ahmady SH (2019) A Phytochemical Study on Olea europaea L. Olive leaf extract (cv. Koroneiki) growing in Egypt. Arch Pharm Sci Ain Shams Univ 3(1):99–105

Khayyal MT, El-Ghazaly MA, Abdalhaim DM, Nokpani SN, Kreuter MH (2002) Blood pressure lowering effect of an olive leaf extract (cv. Koroneiki) growing in Egypt. Arch Pharm Sci Ain Shams Univ 3(1):99–105

Kim D, Chun O, Kim Y, Moon H, Lee C (2003) Quantification of the effect of 15 phenolic olive mill wastewater constituents on seed germination and Vitis vinifera metabolism. J Agric Food Chem 51(21):8414–8417

Kirkpatrick LA, Feeney BC (2013) A simple guide to IBM SPSS statistics for version 20.0. student ed. Belmont. Cengage Learning, Wadsworth

Kontou N, Psaltopoulou T, Soupos N, Polychronopoulos E, Xinopoulos K, Balakrishnan N, Read CB, Vidakovic B (2006) Encyclopedia of statistical sciences, 2nd edn. Wiley-Interscience, Hoboken
Kovácik J, Klejdus B, Babula P, Jarolíková M (2014) Variation of antioxidants and secondary metabolites in nitrogen-deficient barley plants. J Plant Physiol 171(3–4):260–268

Kwan HY, Chao X, Su T, Fu X, Tse AKW, Fong WF (2017) The anticancer and anti-obesity effects of Mediterranean diet. Crit Rev Food Sci Nutr 57(1):82–94

Leung KT, Mok IAC, Suen SN (1992) Polynomials and Equations. Hong Kong University Press, Hong Kong (ISBN 9789622092716)

Marinova D, Ribarova F, Atanassova M (2005) Total phenolics and total flavonoids in Bulgarian fruits and vegetables. J Univ Chem Technol Metall 40(3):255–260

Menéndez JA, Vazquez-Martín A, Oliveras-Ferreros C, García-Villalba R, Carrasco-Pancorbo A, Fernandez-Gutierrez A, Segura-Carretero A (2008) Analyzing effects of extra-virgin olive oil polyphenols on breast cancer-associated fatty acid synthase protein expression using reverse-phase protein microarrays. Int J Mol Med 22(4):433–439

Micol V, Caturla N, Pérez-Fons L, Más V, Pérez L, Estepa A (2005) The olive leaf extract exhibits antiviral activity against viral haemorrhagic septicemia rhabdovirus (VHSV). Antivir Res J 66(2–3):129–136

Mohamed ME, Saad El Din I (2002) Olive cultivation and production. Horticulture Research Institute, Bulletin no. 720

Molyneaux PH (2004) The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26(2):211–219

Omar SH (2010) Oleuropein in olive and its pharmacological effects. Sci Pharm 78(2):133–154

Omwamba M, Li F, Sun G, Hu Q (2013) Antioxidant effect of roasted barley (Hordeum vulgare L.) grain extract towards oxidative stress in vitro and in vivo. Food Nutr Sci 4(8):139–146

Pereira AP, Ferreira IC, Marcelino F, Valentao P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007) Phenolic compounds and antimicrobial activity of olive (Olea europaea L. cv. Cobranca) leaves. Molecules 12(8):1153–1162

Pieroni A, Heimler D, Pieters L, Poel VB, Vlietinck AJ (1996) In vitro anti-complementary activity of flavonoids from olive (Olea europaea L.) leaves. Pharmazie 51(10):765–768

Piroddi M, Albini A, Fabiani R, Giovannelli L, Luceri C (2016) Nutrigenomics of extra-virgin olive oil: a review. BioFactors (oxford-England) 43(1):17–41

Pucci C, Martinelli C, Ciofani G (2019) Innovative approaches for cancer treatment: current perspectives and new challenges. Ecanermedicalscience 13:961

Sánchez-Gutiérrez M, Bascón-Villegas I, Rodríguez A, Pérez-Rodríguez F, Fernández-Prior A, Rosal A, Carrasco E (2021) Valorisation of Olea europaea L. olive leaves through the evaluation of their extracts: antioxidant and antimicrobial activity. Foods 10(966):1–19

Shamshoum H, Vlavcheski F (2017) Anticancer effects of oleuropein. BioFactors 43(4):517–528

Sharma N, Sharma O, Garg N (2021) Phytochemical and pharmacology of Hordeum vulgare linn: a review. World J Pharm Med Res (wjpmr) 7(8):337–342

Sirianni R, Chimento A, De Luca A, Casaburi I, Rizza P (2010) Oleuropein and hydroxytyrosol inhibit MCF-7 breast cancer cell proliferation interfering with ERK1/2 activation. Mol Nutr Food Res 54(6):833–840

Skhean P, Storeng R, Scudiero D, Monks A, McMahon J (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 82(13):1107–1112

Skrget M, Kotnik P, Hadolin M, Hra AR, Simon M, Knez Z (2005) Phenols, proanthocyanidins, flavonoids and flavonol in some plant materials and their antioxidant activities. Food Chem 89(2):191–198

Somova LI, Shode FO, Mipando M (2004) Cardiotonic and antidysrhythmic effects of oleanolic and ursolic acids, methyl maslinate and uvaol. Phytomedicine 11(2–3):121–129

Talib WH, Alsalahat I, Daoud S, Abutayeh RF, Mahmod AI (2020) Plant-derived natural products in cancer research: extraction, mechanism of action, and drug formulation. Molecules 25:5319

Tori J, Markovi AK, Brala CJ, Barbari M (2019) Anticancer effects of olive oil polyphenols and their combinations with anticancer drugs. Acta Pharm 69(4):461–482

Tori J, Brozovi A, Loncar MB, Brala CJ, Markovi AK, Benci D, Barbari M (2020) Biological activity of phenolic compounds in extra virgin olive oils through their phenolic profile and their combination with anticancer drugs observed in human cervical carcinoma and colon adenocarcinoma cells. Antioxidants 9(5):453

Wang L, Geng C, Jiang L, Gong G, Liu D, Yoshimura H, Zhong L (2008) The anti-atherosclerotic effect of olive leaf extract is related to suppressed inflammatory response in rabbits with experimental atherosclerosis. Eur J Nutr 47(5):235–243

Yan CM, Chai EQ, Cai HY, Miao GY, Ma W (2015) Oleuropein induces apoptosis via activation of caspasces and suppression of phosphatidylinositol 3-kinase/protein kinase B pathway in HepG2 human hepatoma cell line. Mol Med Rep 11(6):4617–4624

Yang J, Chen J, Zhao Z, Mao L (2021) Effects of drying processes on the antioxidant properties in sweet potatoes. Agric Sci China 9(10):1522–1529

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