Antimicrobial, antioxidant and anti-EBV activities of olive leaf (*Olea Europea*) extracts from Tunisia

Bochra Gargouri

Universite de Sfax Institut Superieur de Biotechnologie de Sfax

Ichrak Ben Amor (✉ benamor.ichrak@gmail.com)

Faculty of sciences Sfax <https://orcid.org/0000-0003-3263-7164>

Khaoula Tlili

Universite de Sfax Institut Superieur de Biotechnologie de Sfax

Imen Kallel

Faculty of Sciences of Sfax

Hammadi Attia

Universite de Sfax Institut Superieur de Biotechnologie de Sfax

Saloua Lassoued

Universite de Sfax Institut Superieur de Biotechnologie de Sfax

Research

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Abstract

Background: Olive tree leaves (*Olea europaea*) are widely used in traditional medicine in the Mediterranean Region. Interest in the olive leaf and its chemical constituents has recently been increasing. Its benefits, however, have been known for centuries, and it has been traditionally used to prevent and treat different diseases. The aim of this study was the determination of antimicrobial, antioxidant activities and anti-EBV effects of olive leaves. **Methods :** The present study was focused on extraction of leaves of *olea europea* cultivated and wild type from two different geographical tunisian zone: Sfax and El Kef, and the determination of antimicrobial activities against Gram- and Gram+ bacteria and fungi. Olive leaves was analyzed for antioxidant power in chemical system by the DPPH test and the chemiluminescence and in biological system by lipid peroxydation tests (MDA and DC) in Raji cells lines. The antiviral (anti-EBV) effects of olive leaves were assessed using indirect immunofluorescence technique. **Results:** The antioxidant effect was studied by chemiluminescence where a significant decrease on reactive oxygen species production was observed after leaves olive extract treatment. Antioxidant activities were studied by direct reactive oxyden species assay using chemical systems. The results very interesting for both wild type and cultivated in the two regions since we obtained IC50s of the order of 20.79 µg / ml (cultivated sfax) with DPPH scavenging radical. The best result obtained when used olive leaves extract cultivated from Sfax. Our results showed a protection against oxidative stress, highlighted by a significant decrease in MDA and DC levels ($p < 0.05$). Also, a significant antiviral effect of leaves of olive cultivated in Sfax against Epstein-Barr virus (EBV) was determined. **Conclusion:** The olive leaves extracts analyzed in this work showed a potent antimicrobial activity, an antioxidant activity was expressed by a significant decrease in the production of ERO in the Raji cell line and an anti-EBV effect.

Background

Herbal medicine is diseases treatment or prevention through the use of vegetable products obtained by extraction. It is part of alternative medicine which is rich thanks to the inexhaustible mine of observations accumulated after centuries. In their quest for the new molecule, their research focuses on plant substances [1, 2]. Olive leaf extract is well known for its broad health benefits. They are well known for their beneficial effects on metabolism when used as a traditional herbal drug. In Tunisia, olive culture is one of the most important agricultural activities. Olive plantations count about 57 millions trees, where the Chemlali olive is the most widespread and dominant olive cultivar [3]. The olive tree has two species: the cultivated varieties which is the most widespread, and the wild species growing in forests and mountains.

Recently, antioxidant, hypoglycemic, antihypertensive, antimicrobial, and antiatherosclerotic effects of olive leaves have been reported in various studies [4, 5, 6]. The plant extract from olive leaves was reported to have anticancer and antiinflammatory properties [7]. The olive (*Olea europaea* L) leaf is known to be resistant in nature to microorganisms and insect attack, and much research has focused on the antimicrobial activity of compounds contained in olives and olive oil [8]. According to studies, the

antioxidant and antimicrobial activity of olive leaf extracts depended on the varieties and the geographical areas.

On the other hand, Micol et al [9] have shown that olive leaf extracts have antiviral activity against herpes simplex virus (HSV). However the effect against the Epstein Barr virus or against the infection by this virus have not been studied. Indeed, several studies have shown the presence of a state of oxidative stress in cell cultures of positive EBV lines [10, 11, 12]. Similarly, more and more recent studies show that the induction of EBV lytic cycle seems to be involved in the genesis of a state of oxidative stress in vitro [13, 14].

The aim of this study was to realize extraction of olive leaves from two different regions (Sfax and ElKef) by maceration, to investigate the antimicrobial, antioxidant and anti-viral activities. The study of antimicrobial effect was realized by using eight bacterial strains and eight fungal strains. The antioxidant power of our extract was studied in a chemical system, by the DPPH scavenging radical and chemiluminescence technique, then in a biological system using Raji cells. The antiviral effect of olive leaves cultivated from Sfax extract against Virus Epstein Barr (EBV) was determined using the indirect immunofluorescence method.

Materials And Methods

Cell lines and culture conditions

HeLa is a transformed line expressing the HPV18 virus (human Papiloma virus) [15].

Raji is a human Burkitt's lymphoma-derived cell line. As an EBV-positive cell line, the early stage of the viral cycle can be induced after treatment with chemical products, but viral DNA synthesis is completely inhibited; therefore, it is impossible for this cell line to produce viral particles [16,17,14].

HeLa and Raji cell lines were grown in RPMI 1640 medium (Gibco) supplemented with 12 % fetal calf serum (FCS) and 2 mM L-glutamine in tissue culture flasks (Nunc). They were passaged twice a week and kept at 37°C in a humidified atmosphere of 95 % air and 5 % CO₂.

Plant sampling and preparation for extract

The samples of the olive leaves used were collected during the month of october from two different geographical areas (El Kef and Sfax). Our choice was directed towards the most important olive variety : Chemlali (cultivated and wild varieties).

the wild and cultivated olive leaves are dried in ambient air, protected from the light, for 3 weeks, then ground into an electric grinder. The extraction is carried out using a mixture of water / ethanol (v/v) solvents, by simple maceration, for 24 hours, with gentle stirring. Finally, the freeze-dried products are dissolved in 2 ml of dimethylsulfoxide (DMSO) and stored at -20°C until further use.

Antimicrobial activity

In order to evaluate the antimicrobial activity, eight bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella enteritidis* and *Klebsiella pneumoniae*: (Gram- bacteria); *Staphylococcus aureus*, *Listeria monocytogenes*, *Bacillus cereus* and *Micrococcus luteus*: (Gram+ bacteria) and eight fungal strains (*Rhizopus nigricans*, *Fusarium oxysporum*, *Alternaria alternata*, *Pythium aphanidermatum*, *Fusarium culmorum*, *Botrytis cinerea*, *Fusarium graminearum* and *Aspergillus flavus*) were used. For the bacterial culture, 35 g of dehydrated Mueller-Hinton medium (64884) are dissolved in 1 l of distilled water. The solution is stirred slowly until the powder is completely dissolved and then heated in a water bath. After autoclaving for 15 min at 121 ° C, the medium is poured into sterile petri dishes with a thickness of 4 mm agar. The boxes are then allowed to cool on a cold surface until solidification. Colonies isolated from the different bacterial strains to be tested already in culture are removed using a sterile loop and then quickly suspended in the tube containing the subculture medium. A sterile swab is then dipped into the tube containing the bacterial culture obtained to seed the bacteria on the prepared petri dishes. After inoculation, 50 µl of each extract to be studied are deposited on the agar. Each test is done in "duplicate". The dishes are finally incubated for 24 h at 37 ° C. The antibacterial activity results in the presence of a halo of transparency around the deposited drop indicating the inhibition of bacterial growth by the extract.

For fungi, the same methodology is applied except that the culture medium used is the malt extract and the incubation is at 25 ° C for 48 h.

Chemical System

2,2-Diphenyl- 1-picrylhydrazyl (DPPH) free radical scavenging activity assay

The antioxidant activity of extract was evaluated by monitoring its ability in quenching the stable free radical DPPH. Different aliquots from stock solution (200 µl in 1 ml MeOH) of extract were mixed with 500 µl of 0.2 mM diphenylpicrylhydrazine (DPPH) and the final volume brought to 1 ml. The mixture was shaken vigorously and allowed to reach a steady state at room temperature for 1 h. Staining of DPPH was determined by the absorbance measuring at 517 nm with a Beckman spectrophotometer [18]. The DPPH radical scavenging activity was calculated according to the following equation:

$$I \% = \frac{A_0 - A_1}{A_0} \times 100$$

A₀: The absorbance of the total DPPH (blank, without extract)

A₁ ; The absorbance of the sample.

Chemiluminesce Assay

This technique is a chemical reaction that emits energy in the form of light in the presence of a regulating probe with the reactive forms of oxygen. Luminol (3-aminophthalhydrazide), will be oxidized to give 3-aminophthalate, will return to the basic state by emitting light detected by the luminometer [19]. In each well of a chemiluminescence plate, the following compounds were added: olive leaves extracts at different concentrations, 50 µl luminol, 20 µl FeSO₄ and 20 µl H₂O₂. Indeed, the two chemical agents, H₂O₂ and FeSO₄, were used to induce the Fenton reaction. FeSO₄ produces different types of ROS (superoxide anion, hydrogen peroxide and hydroxyl radical), by reacting ferrous ion (Fe²⁺) with oxygen. H₂O₂ is not toxic, but in the presence of low-dose transition metal anion, hydrogen peroxide can interact with the superoxide anion to produce, according to the Fenton reaction, the hydroxyl radical that is very active [14].

MTT cell proliferation assay

The MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) cell proliferation assay measures the cell proliferation rate and conversely, the reduction in cell viability when metabolic events lead to apoptosis or necrosis. The yellow compound MTT (Sigma) is reduced by mitochondrial dehydrogenases to the water insoluble blue formazan compound, depending on the viability of the cells. Cells (12000 cells/ml) were grown on microtiter plate in 96 well microplates with serial dilutions of extract. 72 h later, 20 µl of a MTT solution were added in each well. The plate was incubated for 4 h at 37°C in a CO₂ incubator. After incubation, 180 µl of medium were removed from each well and 180 µl of DMSO/methanol (50:50) were added to each sample. The preparations were mixed thoroughly on a plate shaker with the cells containing formazan crystals. When all the crystals were dissolved, absorbance was measured at 570 nm with a microplate reader (Elx 800 microplate reader).

Biological System

Preparation of cell lysate

Raji cell lines was treated simultaneously with [8 nM] TPA and extracts for 30 min. At the end of the incubation period, the cells were collected. After centrifugation for 10 min at 1000 rpm, the cell pellet is washed once with PBS (1 X) and then centrifuged for 10 min at 1000 rpm. The pellet was resuspended in 1 ml H₂O (deionized water) and stored at -20 ° C until further use. Cell lysis is carried out by 10 cycles of sonication for 20 s at 37% (Sonisc, vibracell).

Protein determination

Proteins were determined using the Protein Assay Kit from Bio-Rad (France) and bovine serum albumin served as the standard [14].

MDA Determination

MDA determination was performed by the thiobarbituric acid reactive species assay. 350 µl of Raji cell lysate are added to 700 µl reagent TBA / TCA (thiobarbituric acid / trichloroacetic acid) (15% trichloroacetic acid, 0.8% TBA, 0.25 N HCl). The mixture was heated at 95°C for 15 min to form MDA-TBA adduct. Optical density was measured with a spectrophotometer (Biochrom, Libra S32) at 532 nm. Values were reported to a calibration curve of 1,1,3,3-tetraethoxypropane [19].

Conjugated Dienes

Conjugated diene level was evaluated as described by Kurien and Scofield [20] with modification. 25 µl of Raji cells lysate were extracted with 3 ml chloroform/methanol (2:1, v/v). After centrifugation at 3000 rpm for 15 min, 2 ml of organic phase was transferred into another tube and dried at 45°C. The dried lipids were dissolved in 2 ml of methanol and absorbance at 233 nm was determined.

The Anti EBV study using Indirect Immunofluorescence

The cells are cultured at a concentration of 3×10^6 cells / ml for 48 h. After the addition of TPA [8 nm] and simultaneous treatment with plant extract for 30 min, the cells were incubated for 48 h and then centrifuged for 5 min at 1000 rpm. The primary antibody (serum of nasopharyngeal cancer NPC patients) are diluted 1/500 in PBS (1X) and added to the cell pellet. The cells were then incubated 20 min at 4 ° C. Two washes are carried out with 500 µl of PBS (1X) after centrifugation.

The antihuman IgG secondary antibody was diluted 1/500 in PBS (1X) and then added to the cell pellet. After incubation for 20 min at 4 °C in the dark, the cells were centrifuged and washed once with PBS (1X). The final pellet obtained after centrifugation, is suspended in 50 µl of PBS, 25 µl of the suspension is deposited between slide and cover slip. Finally the slides are observed under the microscope equipped with an epifluorescence device to the objective 40.

Statistical analysis

The one-way analysis of variance (ANOVA) was performed at the level of $p < 0.05$ to evaluate the significance of differences between mean values. Statistical analysis was performed using SPSS statistical software.

Results

Antimicrobial activity

Antibacterial activity

The antibacterial activity results in the presence of a halo of transparency around the deposited drop indicating the inhibition of bacterial growth by the extract.

The diameter of each halo, we have checked the antibacterial power of the extracts. Indeed, the larger of the diameter, the greater the ability of bacterial inhibition by an extract is important. Tables 1 represent the results obtained in our study. These tables make it possible to compare the inhibitory power of the various extracts between them on the growth of the eight strains.

Our results show that our extracts of wild and cultivated olive leaves from two geographical zones (Sfax, El Kef), have a significant antibacterial activity against Gram- and Gram+ bacteria.

In general, extracts of wild olive leaves have significant antibacterial potential against Gram- bacteria than extracts of cultivated olive leaves. On the other hand, the extracts of the cultivated olive leaves Sfax and El Kef, highlighted significant antibacterial activity against the Gram-bacteria than the extracts of the wild olive leaves Sfax and El Kef. The olive leaf extract wild type Sfax has the best antibacterial activity, since it presents an activity against all Gram-.

Antifungal activity

The results obtained concerning antifungal activity are grouped in the table 2. Our results showed that wild and cultivated leaf extracts from both geographical areas (Sfax and El Kef) exhibited an antifungal activity. Indeed, this activity is observed on four fungi: *Rhizopus nigrians*, *Botrytis cinerea*, *Alternaria alternata* and *Aspergillus flavus*. The Sfax cultivated olive leaf extract exhibited the best antifungal activity.

Chemical System

DPPH radical scavenging activity assay

The results of the antioxidant activity showed that the four extracts of wild and cultivated olive leaves had a significant ($p < 0.05$) anti-radical activity (Figure 1). This antioxidant activity is dose dependent.

The results obtained (figure 2) show that the cultivated variety has IC₅₀s of the order of 20.79 µg / ml (cultivated Sfax) and 21.92 µg / ml (cultivated EL Kef) which are lower than that of the synthetic antioxidant (BHT) which has an IC₅₀ equal to 27.31 µg / ml. However, for the wild olive leaf extract sfax has an IC₅₀ lower than that of BHT of the order of 21.54 µg / ml while the extracts of wild leaves EL Kef have a IC₅₀ higher than that of BHT. The cultivated variety has the significant highest antioxidant activity, and that the wild variety has the lowest antioxidant activity compared to the BHT standard.

Chemiluminescence assay

The chemiluminescence technique was used to determine the potency of our olive leaf extracts to neutralize reactive oxygen species and the product of the Fenton reaction to the OH radical. Our results showed that the four extracts had significant antioxidant activity ($p < 0.05$) (Figure 3). Depending on these results, the antimicrobial and antioxidant activities of olive leaves extract were more observed with the extract cultivated from Sfax which will be used to study antioxidant activity on Raji cells lines and anti EBV activity.

MTT cell proliferation assay

The cytotoxic effect on Raji cell lines was studied by the MTT test. 24×10^6 cells / well were cultured in 96-well plates for 48 h, in the presence and absence of the different concentrations of olive leaves cultivated from Sfax (OLCS). The percentage of cytotoxicity was calculated.

Our results showed a cytotoxic effect of OLCS on the Raji cell line according to the concentrations used. The concentration of the extract selected for further work is that which has a percentage of cytotoxicity less than 50%. This non-cytotoxic concentration is 0.3 mg / ml (figure 4).

Biological System

MDA Determination

After TPA [8 nM] treatment, there was a significant increase in MDA levels in the Raji cell line, compared to untreated cells, indicating the presence of a state of oxidative stress as a consequence of EBV lytic cycle induction ($p < 0.001$).

Treatment of Raji cells simultaneously by TPA and OLCS [0.3 mg / ml] induces a significant decrease in MDA levels ($p < 0.05$) (figure 5).

Conjugated Dienes

Treatment of Raji cells lines with TPA [8 nM] induced a significant increase in DC levels compared to untreated cells ($p < 0.001$). Treatment of the Raji cells lines simultaneously with TPA and OLCS induces a significant decrease in DC levels compared to untreated cells ($p < 0.05$) (figure 6).

Anti EBV study

The results (figure 7) obtained show a protective effect of OLCS against viral reactivation in the Raji cell line, demonstrated by the decrease in the percentage of fluorescence in the cells treated with this extract compared to the positive control (table 3).

Discussion

Olive leaves have very good biological activity ; antihypertensive, anti-atherogenic, anti-inflammatory, hypoglycemic and hypocholesterolemic effects [21, 22]. The olive leaves extracts analyzed in this work showed a potent antimicrobial activity. Our results are in agreement with that of Djenane et al [23], who showed a strong antibacterial activity of olive leaf extracts of the variety chemlali (cultivated variety) against Gram- and Gram + bacteria. Bisignano et al [24] showed antibacterial activity against Gram- and Gram + bacteria, extract of wild olive leaves. Other studies confirmed that the presence of leaf extract from northeast of Portugal in culture medium showed to induce or repress the antimicrobial activity [25]. Olive leaf aqueous extracts were screened for their antimicrobial activity against *B cereus*, *B subtilis*, *S*

aureus (Gram +), E coli, P aeruginosa, K pneumoniae (Gram -) bacteria, and C albicans and C neoformans (fungi) [26]. In opposition other study who demonstrated that Gram + bacteria are more sensitive to plant extracts than Gram- bacteria [27]. The antibacterial activity of our 4 extracts against Gram - bacteria could be explained by the properties of its components, at the same time lipophilic and hydrophilic, such as hydroxytyrosol and oleuropein [28]. The difference in ability to inhibit bacterial growth between extracts is due to differences in composition and also in the concentration of components present in the extracts. In addition, an antimicrobial activity of olive leaves against Staphylococcus aureus [6] and Bacillus cereus, Escherichia coli and Salmonella enteritidis [29] was observed. Furthermore, other study has shown that olive leaves extract showed a potent antibacterial effect against P aeruginosa, K pneumoniae and S aureus [30].

In other hand, our extracts was showed an antifungal power especially for OLCS. Our results are in agreement with the study of Sokmen and Korukluoglu who demonstrated that the olive leaf extracts was screened for their antifungal activity against different strains of fungi example A alternata and A flavus used in our studies [31, 8].

The antioxidant activity of the olive leaf extracts was evaluated by the DPPH radical scavenging assay and the chemiluminescence technique. Our results concerning the antioxidant activity evaluated by the diphenyl picrylhydrazyl (DPPH) radical-scavenging displayed a strong antioxidant activity with $IC_{50} = 20.79$ for OLCS. Our results are in agreement with many studies have shown that olive leaves possesses a strong antioxidant activity in the DPPH radical-scavenging assay [32, 3, 33, 34, 35].

Our results regarding chemiluminescence have shown antioxidant activity of our extracts. Chemiluminescence study was realised for the first time to study antioxidant power of olive leaves extracts.

In our study the OLCS represent the best antibacterial, antifungal and chemical antioxidant activities. It be used to study antioxidant power in Raji cell line after EBV lytic cycle induction.

The antioxidant effect on lipid peroxidation was studied in Raji cell line. Simultaneous treatment of cells with TPA and OLCS causes a significant decrease in MDA and DC production. A study conducted by Dekanski et al [36] documented that MDA concentration is significantly increased in gastric mucosa exposed to ethanol or water immersion and restraint stress. MDA was reduced significantly by pretreatment with 80 mg/kg of olive leaves extracts.

Our finding revealed that OLCS showed an antiviral activity against EBV. This effect is evidenced by the decrease in the percentage of fluorescence after OLCS treated cells compared to the untreated cells. According to old studies, the extract of the olive leaves has an activity against the virus ILTV [37]. On the other hand, Vicente Micol et al have shown that the oleuropein fraction of the olive leaf extract has activity against the VHSV virus [9]. Our results are in agreement with those of Lee-Huang et al., Showing that extracts prepared from olive leaves have an inhibitory effect on the fusion and integration of HIV into

human cells [38]. Others studies confirmed the antiviral effect of OLHE highlighted a significant antiviral activity against HSV-1 [39].

Conclusion

Olive-leaf is one of the most common, traditional herbal used among Mediterranean people to cure certain diseases. Accordingly, olive leaves extract exhibited strong anti-bacterial activity against bacteria Gram – and Gram +. Also an antifungal effect was detected specially against *Rhizopus nigrians*, *Botrytis cinerea*, *Alternaria alternata* and *Aspergillus flavus*. Furthermore, the analysis of olive leaves extract revealed an anti-oxidant activity and protection against lipid peroxidation by measuring the levels of MDA and DC in Raji cell line. In addition, the leaves of *oleo europea* cultivated from Sfax showed an anti-viral effect against EBV.

Abbreviations

CD: Conjugated dienes; DPPH 1,1-Diphenyl-2-picrylhydrazyl ; EBV : Epstein–Barr virus ; MDA : Malondialdehyde ; OD: Optical density ; OLCS: Olive Leaves Cultivated in Sfax ; OLWS: Olive Leaves Wild type in Sfax ; OLCK: Olive Leaves Cultivated in Kef ; OLWK: Olive Leaves Wild type in Kef ; TPA:12-O-Tetradecanoylphorbol-13-acetate; TBA: Thiobarbituric acid; ROS : Reactive oxygen species ; PBS: phosphate buffer saline.

Declarations

Availability of data and material

The dataset supporting the conclusions of this article is included within the article.

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable

Authors' contributions

Bochra Gargouri and Ichrak Ben Amor are equally contributed to this work. All authors conceived the study, and participated in its coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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Affiliations

1. Unité de Biotechnologie et Pathologies(LR11ES45), Institut Supérieur de Biotechnologie de Sfax, University of Sfax, Sfax, Tunisia : Bochra Gargouri, Ichrak Ben Amor, Khaoula Tlili, Hammadi Attia and Saloua Lassoued
2. Laboratoire de recherche Toxicologie- Microbiologie Environnementale et Santé, Faculté des Sciences de Sfax, Sfax, Tunisie : Imen Kallel

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Tables

Due to technical limitations, the tables are only available as a download in the supplemental files section.

Figures

Figure 1: Anti-radical activity against DPPH radical in percentage polyphenolic extracts of wild olive leaves and cultivated from different geographical areas and BHT standard

OLCS: Olive Leaves Cultivated in Sfax

OLWS: Olive Leaves Wild type in Sfax

OLCK: Olive Leaves Cultivated in Kef

OLWK: Olive Leaves Wild type in Kef

Figure 1

Anti-radical activity against DPPH radical in percentage polyphenolic extracts of wild olive leaves and cultivated from different geographical areas and BHT standard

OLCS: Olive Leaves Cultivated in Sfax
 OLWS: Olive Leaves Wild type in Sfax
 OLCK: Olive Leaves Cultivated in Kef
 OLWK: Olive Leaves Wild type in Kef

Figure 2: Concentrations responsible for 50% inhibition of the radical DPPH (IC50).

Polyphenolic extracts of wild and cultivated olive leaves from different geographical areas and BHT standard.

OLCS: Olive Leaves Cultivated in Sfax

OLWS: Olive Leaves Wild type in Sfax

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Figure 2

Concentrations responsible for 50% inhibition of the radical DPPH (IC50). Polyphenolic extracts of wild and cultivated olive leaves from different geographical areas and BHT standard. OLCS: Olive Leaves Cultivated in Sfax OLWS: Olive Leaves Wild type in Sfax OLCK: Olive Leaves Cultivated in Kef OLWK: Olive Leaves Wild type in Kef

Figure 3: Anti-radical activity (against reactive oxygen species) in percentage polyphenolic extracts of wild olive leaves and cultivated from two different geographical areas.

OLCS: Olive Leaves Cultivated in Sfax

OLWS: Olive Leaves Wild type in Sfax

OLCK: Olive Leaves Cultivated in Kef

OLWK: Olive Leaves Wild type in Kef

Figure 3

Anti-radical activity (against reactive oxygen species) in percentage polyphenolic extracts of wild olive leaves and cultivated from two different geographical areas. OLCS: Olive Leaves Cultivated in Sfax
OLWS: Olive Leaves Wild type in Sfax OLCK: Olive Leaves Cultivated in Kef OLWK: Olive Leaves Wild type in Kef

Figure 4: OLCS cytotoxicity Study in Raji cell line

Figure 4: 12000 Raji cells / well are cultured in 96 well plates in the presence of increasing concentrations of the OLCS. The percentage of cytotoxicity is evaluated by the MTT test.

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OLCS cytotoxicity Study in Raji cell line 12000 Raji cells / well are cultured in 96 well plates in the presence of increasing concentrations of the OLCS. The percentage of cytotoxicity is evaluated by the MTT test.

Figure 5: Study of OLCS effects on MDA production in Raji cell line (C-: untreated cells, C+: cells treated with TPA [8nm]).

Figure 5: 3×10^6 cells are cultured in the presence and absence of TPA and OLCS simultaneously at a non-cytotoxic concentration (0.3 mg / ml). After washing with PBS, the cells are cultured for 48 h. The level of MDA produced is evaluated by the TBARS technique. The results are expressed in mmoles / mg of proteins (*: $p < 0.05$).

Figure 5

Study of OLCS effects on MDA production in Raji cell line (C-: untreated cells, C+: cells treated with TPA [8nm]). 3×10^6 cells are cultured in the presence and absence of TPA and OLCS simultaneously at a non-cytotoxic concentration (0.3 mg / ml). After washing with PBS, the cells are cultured for 48 h. The level of MDA produced is evaluated by the TBARS technique. The results are expressed in mmoles / mg of proteins (*: $p < 0.05$).

Figure 6: Study of OLCS effect on the DC production on Raji cell line (C-: untreated cells, C +: cells treated with TPA [8nm])

Figure 6 : 3×10^5 cells are cultured in the presence and absence of TPA and OLCS simultaneously, at a non-cytotoxic concentration (0.3 mg / ml). After washing with PBS, the cells are cultured for 48 hours. The level of DC produced is evaluated by measuring the OD at 233 nm (*: $p < 0.05$).

Figure 6

Study of OLCS effect on the DC production on Raji cell line (C-: untreated cells, C +: cells treated with TPA [8nm]) * 10^5 cells are cultured in the presence and absence of TPA and OLCS simultaneously, at a non-cytotoxic concentration (0.3 mg / ml). After washing with PBS, the cells are cultured for 48 hours. The level of DC produced is evaluated by measuring the OD at 233 nm (*: $p < 0.05$).

Contrôle positif

Contrôle négatif

OLCS

Figure 7 : Study of the antiviral effect of OLCS in the Raji cells line (C+: cells treated with TPA, C-: HeLa cell)

Figure 7

Study of the antiviral effect of OLCS in the Raji cells line (C+: cells treated with TPA, C-: HeLa cell)

Supplementary Files

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