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Antioxidant properties and anticancer activity of *Olea europaea* L. olive and *Ficus carica* fruit extracts against pancreatic cancer cell lines

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ABSTRACT

Olea europaea (OFE) and *Ficus carica* (FFE) extracts have shown anticancer activity. In the currents report, we aim to evaluate the phytochemical composition, antioxidant properties and anticancer activity of OFE and FFE against PANC-1 and AsPC-1 pancreatic cancer cell lines. OFE possessed higher phenolic, flavonoid content and antioxidant ability than FFE. OFE and FFE induced a cytotoxic effect and their combination resulted in an increased cytotoxicity. OFE showed an antiproliferative effect while FFE had no effect. Combination of the two extracts resulted in an antiproliferative effect similar to that of OFE. OFE showed an anti-migratory effect, while FFE exhibited a promigratory effect. Interestingly, combination of the two extracts resulted in an anti-migratory effect similar to that caused by OFE. In view of the findings of this study, these extracts could be considered potential sources of natural compounds with antioxidant and anti-pancreatic cancer effects. The combination of these extracts should be further investigated in the context of pancreatic cancer.

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1. Introduction

Medicinal plants have shown great promise in the fight against cancer, with many studies demonstrating their potent anticancer properties. Several naturally occurring plant-based compounds, such as curcumin, resveratrol, and quercetin, have exhibited anticancer effects and are being investigated as potential adjuvant chemotherapy agents (Greenwell and Rahman, 2015; Khan et al., 2019; Mahdavi and Mohammadhosseini 2022). These plant-derived compounds often have lower toxicity towards healthy cells and a tendency to target abnormal or cancerous cells specifically. Many of the drugs currently on the market are structurally similar to natural compounds found in medicinal plants (Khan et al., 2019; Obafemi and Besong, 2023). Herbal compounds possess various beneficial qualities, including antioxidant, anti-inflammatory, antimutagenic, and apoptosis-inducing activities, which aid in the early diagnosis and treatment of cancer. Adequate dietary intake of these herbal products may help prevent and treat cancer by causing cell cycle arrest, inducing apoptosis, regulating carcinogen metabolism and oncogenic expression, inhibiting cell adhesion, proliferation, and migration, and blocking signaling pathways crucial for cancer progression (Obafemi and Besong, 2023). Plants produce a wide variety of secondary metabolites, such as tannins, alkaloids, terpenoids, flavonoids, pigments, and pigment-like molecules, which have been shown to have biological effects on hematopoietic cells, lipids, and cardiovascular systems, as well as anti-inflammatory, anticancer, and contraceptive effects (Mohammadhosseini et al., 2019a; Mohammadhosseini et al., 2019b; Mohammadhosseini et al., 2021).

Pancreatic cancer is one of the most aggressive cancers with poor prognosis and survival despite surgical and adjuvant therapies. Currently, it constitutes the fourth

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cause of cancer mortality in occidental countries (Siegel et al., 2016). Treatment with gemcitabine, a pyrimidine agonist, has been the standard of care for metastatic pancreatic cancer for more than a decade. However, survival for most patients treated with this drug has not improved beyond 6 months (Moore et al., 2007; Park et al., 2021). One of the main problems posed with anticancer drugs is that they target not only tumor cells, but also other cell types, leading to toxicity to both abnormal and normal cells (Baldo and Pham, 2014). Another problem related to anticancer therapy is the development of resistance of cancer cells against anticancer treatments (Bar-Zeevet et al., 2017). This is the case of pancreatic cancer cells, which are highly resistant to current chemotherapy and radiotherapy treatments (Vendrely et al., 2017). Therefore, finding new therapeutic options is urgently needed to help manage this deadly cancer.

Nutrition and diet are good candidate agents for protection against various types of cancer (Kazeminia et al., 2022). Epidemiological studies have shown a reduction in the risk of pancreatic cancer through increased consumption of fruits and vegetables (Zheng et al., 2017; Gianfredi et al., 2022). The Mediterranean diet has been recognized as a healthy diet with a preventive effect against chronic diseases associated with oxidative damage, including cardiovascular disease, diabetes, and cancer (Schwingshackl and Hoffmann, 2014; Saha et al., 2017; Mentella et al., 2019; Guasch-Ferré and Willett, 2021). These effects seem to be mediated by many individual components of this diet, including polyphenolic phytochemicals present in fruits such as figs and olive oil (Owen et al., 2000b; Solomon et al., 2006; Lopez-Miranda, 2010).

The olive tree, *Olea europaea*, produces the olive fruit and is one of the vital components of the Mediterranean diet. Olive and its products have many health benefits, including cancer (Markellos et al., 2022; Romani et al., 2019). Preclinical *in vitro* and *in vivo* studies have demonstrated an antitumoral effect of olive fruit extracts (OFE) against hepatic and colorectal cancer (Maalejet al., 2017; Shirazi et al., 2018; Celano et al., 2019). The components of olives and olive oil that have been most consistently associated with a protective effect on carcinogenesis are vitamin E, squalene, and phenolic compounds (Guo et al., 2018). The most abundant phenolic compounds in olive oil are the lignans (+)-1-acetoxypinoresinol and (+)-pinoresinol, followed by the oleuropein-derived simple phenols hydroxytyrosol and tyrosol and the secoiridoids oleuropein, ligstroside aglycone and their respective decarboxylated dialdehyde derivatives (Owen et al., 2004). In contrast, polyphenols such as the flavonoid luteolin, the phenolic acid gallic acid, the phenolic alcohols hydroxytyrosol and tyrosol and the secoiridoids oleuropein and verbascoside are most abundant in olive fruits (Owen et al., 2004; Dekdouket al., 2015). Previous studies have clearly evidenced an antitumor effect of these phenolic compounds against certain types of cancers, namely breast (Han et al., 2009; González et al., 2013; Carrera- Akl et al. 2014; Sepporta et al., 2014), colorectal (Owen et al., 2000a; Carrera-González et al., 2013; Hormozi et al., 2020), gastric (Kountouri et al., 2009), hepatic (Pei et al., 2016), skin cancer (Carrera-González et al., 2013) and leukemia (Fabiani et al., 2002), and also pancreatic cancer (LeGendre et al., 2015; Goldsmith et al., 2018).

Ficus carica is a species characteristic of the Mediterranean region where its cultivation and use constitute an ancient tradition. Figs are consumed both fresh and dried (Baby and Justin, 2011). Figs are an excellent source of minerals, vitamins, carbohydrates, and dietary fiber, they are fat- and cholesterol-free and rich in several amino acids (Cruz et al., 2022; Olaoluwa et al., 2022; Ijoma et al., 2023; Popwo Tameye et al., 2023). Figs have traditionally been used for their medicinal benefits as laxative and cardiovascular, respiratory, antispasmodic and anti-inflammatory remedies (Guarrera, 2005). However, figs also exhibit anticancer properties (Morovatiet al., 2022). Namely, preclinical *in vitro* and *in vivo* models revealed anticancer activity of *F. carica* latex or fruit extracts (FFE) in relation to several types of cancers, including breast (Ghandehari et al., 2018), colorectal (Soltana et al., 2019), gastric (Hashemi et al., 2011), hepatic (Purnamasariet al., 2019) and, recently, also in relation to pancreatic cancer (Ou et al., 2022). An important organic compound in figs is 2-benzhydrylsulfinyl-*N*-hydroxyacetamide-Na, which is less abundant in other fruits and plants, and exhibits anticancer activity *in vitro* (Al Salman et al., 2020). Moreover, *Ficus carica* is a rich source of polyphenols. Quercetin and quercetin-related compounds are the main phenolic compounds present in this fruit (Khadhraoui et al., 2019; Arvaniti et al., 2019), and are well known to possess antitumor activity against several types of cancer, including pancreatic cancer (Rauf et al., 2018).

Several *in vitro* and *in vivo* preclinical studies have demonstrated varying degrees of efficacy of natural products against pancreatic cancer (Singh et al., 2015), and numerous dietary and pharmacological agents have been proposed as alternative strategies for the prevention and/or treatment of this disease (Dhar et al., 2018; Selvarajoo et al., 2022).

To the best of our knowledge, no report on the effects of a combination of OFE and FFE on pancreatic cancer cells is available in the literature. Keeping this point in view, the present study was conducted to evaluate the *in vitro* antioxidant activities and the cytotoxic, antiproliferative and antimigratory effects of OFE and FFE, alone and in combination, on two pancreatic cancer cell lines.

2. Experimental

2.1. Chemicals and reagents

^{3}H-Thymidine ([methyl-^{3}H]-thymidine, 79 Ci/mmol) was purchased from GE Healthcare GmbH (Freiburg, Germany). Dimethylsulfoxide (DMSO), hydrochloric acid (HCl), triton X-100, trichloroacetic acid (TCA) were purchased from Merck (Darmstadt, Germany). Aluminium chloride ($AlCl_3$), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, Folin-Ciocalteus's phenol reagent, ascorbic acid, sodium carbonate (Na_2CO_3), potassium ferricyanide [$K_3Fe(CN)_6$], 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate ($K_2S_2O_8$),

2,6-di-tert-butyl-4-hydroxy toluene (BHA), butylated hydroxyltoluene (BHT), α-tocopherol, iron (III) chloride ($FeCl_3$), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), antibiotic/antimicotic solution (100 U/mL penicillin, 100 µg/mL streptomycin, and 0.25 µg/mL amphotericin B), Dulbecco's Modified Eagle's Medium-high glucose, fetal bovine serum, *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), RPMI 1640 medium, sodium hydroxide (NaOH) and trypsin-ethylenediamine-tetraacetic acid (EDTA) solution were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extraction and identification of phenolic compounds

2.2.1. Plant material

Fruits of the *F. carica* 'Mkaousi' cultivar were harvested in the Constantine region (Algeria) in September 2017. Olives of the *Chemlal* cultivar of *Olea europaea* were harvested in Algeria in December 2017. The olives were handpicked during the harvest season and were immediately frozen in liquid nitrogen to block enzyme activities. Healthy fruits (100 g), without any physical damage or type of infection, were selected, and lyophilized. A reference specimen for each sample is deposited at the Herbarium of the Faculty of Natural and Life Sciences (Université des Frères Mentouri Constantine, Algeria). The plant samples were authenticated by Mendil, M, Director General of Technical Institute of Fruit Arboriculture and Vine (ITAF), Algeria.

2.2.2. Extraction procedure

The lyophilized fruits of *O. europaea* (100 g) were defatted with hexane, and then extracted for 48 h with $CHCl_3$, and MeOH, successively, to give 28.33, 2.7, and 5.37 g of the respective residues. The polyphenols from lyophilized fruits of *F. carica* were extracted by maceration using ethyl acetate to give 2.07 g. It has been demonstrated that this solvent is better than others to extract phenolics (Fki et al., 2005). Solvent was renewed 3 times and the obtained extracts were dried using a rotary evaporator.

2.2.3. Determination of total phenolic content (TPC)

The TPC of crude OFE and FFE was estimated spectrophotometrically using Folin-Ciocalteu method (Le et al., 2007). In brief, 200 µL of each extract were added to 1 mL of Folin-Ciolcalteu reagent (10% (*w/v*)). After 4 min, 800 µL of sodium carbonate solution (75 g/L) was added. The mixture was allowed to react for 2 h in darkness at room temperature.
After incubation, the absorbance was recorded at 765 nm using UV-Vis spectrophotometer (VIS-7220G). Gallic acid (5-200 µg/mL) was used as reference to establish the calibration curve from which the concentration of polyphenols was calculated, and the results are expressed as TPC (mg EGA/100 g extract).

2.2.4. Determination of total flavonoid content (TFC)

The quantification of TFC was performed by the trichloroaluminum method (Turkoglu et al., 2007). Briefly, 1 mL of $AlCl_3$ (2.0 (*w/v*)) solution was added to 1 mL of OFE or FFE. The mixture was vigorously stirred and incubated for 10 min at room temperature, and then the absorbance of each sample was read at 430 nm. Quercetin (1.25-25 µg/mL) was used to establish the calibration curve used to estimate the concentration of flavonoids found in the crude extracts. The results are given as TFC (mg EQ/100 g extract).

2.3. Antioxidant activity of OFE and FFE

2.3.1. DPPH• (1,1-DIphenyl-2-picrylhydrazil) scavenging assay

The antiradical activity of OFE and FFE was evaluated by the free radical DPPH• assay (Blois,1958). In brief, 25 µL of different dilutions of the extracts or standards BHA (butylated hydroxyl anisole) and BHT (butylhydroxytoluene) were added to 975 µL of DPPH (0.025 mg/mL) prepared in methanol. The obtained mixture was incubated for 30 min in obscurity. Then, the absorbances were measured using a UV-Vis spectrophotometer at 517 nm and the percentage of DPPH• free radical-scavenging activity of each extract was calculated as follows, where I(%) is the percentage of inhibition and A_C and A_S are the absorbances of the control and the test sample, respectively (Eqn. 1):

$$I(\%) = [(A_C - A_S)/A_C] \times 100 \quad \text{(Eqn. 1)}$$

The results were compared to the standard antioxidant BHA and BHT.

2.3.2. ABTS•+ (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) scavenging assay

The ABTS•+ scavenging activity of OFE and FFE was performed spectrophotometrically (Re et al., 1999). 2 mL of the diluted ABTS cation solution was added to 1 mL of sample solution: Extracts or standards (BHT and BHA) at different concentrations (0.0156-1 mg/mL). After 30 min of incubation, the absorbance was measured at 734 nm and the percentage of ABTS scavenging effect of each extract was calculated using Eqn. 1, where I(%) is the percentage of inhibition and A_C and A_S are the absorbances of the control and the test sample, respectively. The results were compared to the standard antioxidant BHA and BHT.

2.3.3. Cupric acid reducing antioxidant capacity (CUPRAC)

Fifty µL of a solution of copper(II) chloride (10 mM) were added to 50 µL of the neocuprine solution (7.5 mM) and 60 µL of ammonium acetate buffer solution (1 M, pH = 7.0). Different concentrations of OFE, FFE and standards (BHT and BHA) were added to the initial mixture to make a final volume of 200 µL. The samples were shielded from light and the absorbance was measured at 450 nm after 1 h of incubation. The results were calculated as A 0.5 (µg/mL) (the absorbance whose inhibition is equivalent to 50%), and the reduction capacity of the extracts was compared with those of BHA and BHT as

standards (Apak et al., 2004).

2.3.4. Reducing power assay

The reducing power of OFE and FFE were examined according to the previous method (Oyaizu, 1986). Accordingly, 100 µL of sample solution (extracts or references) prepared at different concentrations were added to 0.5 mL of phosphate buffer (0.2 M pH 6.6) and 0.5 mL of potassium ferricyanide (1.0 (*w/v*)). The mixture was incubated at 50 °C for 20 min. Then, 500 µL of CCl_3COOH (10% (*w/v*)) was added to the mixture, which was centrifuged (10 min/3000 rpm). A volume of 500 µL of the supernatant solution was mixed with 500 µL of distilled water and 125 µL of $FeCl_3$ (1.0% (*w/v*)) freshly prepared. The absorptions were read at 700 nm and the results were calculated as A 0.5 (µg/mL) (the absorbance whose inhibition is equivalent to 50%). The reducing power of the various extracts was compared with those of ascorbic and tannic acids as standards.

2.4. Anticancer effect OFE and FFE

2.4.1. Cell culture and treatments

PANC-1 cell line (human pancreatic epithelioid carcinoma cell line) and AsPC-1 cell line (human pancreatic ascites adenocarcinoma cell line) were obtained from the American Type Culture Collection (ATCC) and used between passage numbers 36-38. Cells were maintained in a humidified atmosphere of 5% CO_2-95% air and were grown in Dulbecco's modified Eagle's medium supplemented with 4.5 g/L glucose, 2 mM L-glutamine, 10 mM sodium bicarbonate, 10 (*v/v*%) heat-inactivated fetal bovine serum and 1.0 (*v/v*%) antibiotic/antimycotic (PANC-1 cells) or in RPMI 1640 supplemented with 2 mM of L-glutamine, 10 mM of sodium bicarbonate, 1 mM of sodium pyruvate, 10 (*v/v*%) heat-inactivated fetal bovine serum and 1.0 (*v/v*%) antibiotic/antimycotic (AsPC-1 cells). Culture medium was changed every 2-3 days and the culture was split every 7 days. For sub-culturing, the cells were removed enzymatically (0.25 (*w/v*%) trypsin-EDTA, 37 °C), split 1:4 (PANC-1) or 1:3 (AsPC-1) and sub-cultured in plastic culture dishes (21 cm^2; Ø 60 mm, TPP®, Trasadingen, Switerzland). For determination of migration, viability and cell proliferation, cells were seeded on 24-well plastic cell culture dishes (2 cm^2; Ø 16 mm; TPP®, Trasadingen, Switerzland) and were used after 7 (PANC-1 cells) or 11 days (AsPC-1 cells) (90% confluence). The OFE and FFE to be tested were dissolved in DMSO. The final concentration of the solvent in the culture medium was 1.0 (*v/v*%). Controls for the extracts were run in the presence of this solvent.

2.4.2. Determination of cell viability

Cell viability was determined with the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Briefly, cells were exposed to OFE and/or FFE (or to the solvent) for 24 h. After the treatment period, 30 µL of MTT solution (5 mg/mL) was added to each well and the cells were further incubated for 3 h at 37 °C. Afterwards, the MTT solution was removed and the cells were lysed by the addition of 200 µL of DMSO followed by plate shaking for 10 min at room temperature. Optical density (OD) for the solution in each well was determined at both 540 and 660 nm. The OD at 660 nm corresponds to unspecific light absorption and was subtracted from the OD at 540 nm to give the OD value specific to formazan crystals derived from MTT cleavage, which is proportional to the number of viable cells with active mitochondria.

2.4.3. Determination of cell proliferation

Cell proliferation rate was determined by the ^{3}H-thymidine incorporation assay. Briefly, cells were exposed to OFE and/or FFE (or the solvent) for 24 h, being incubated with^{3}H-thymidine 0.025 µCi/mL in the last 5 h of treatment. After removal of excess ^{3}H-thymidine by a wash with 300 µL of 10% TCA (*w/v*) for 1 h at 4 °C, drying for 30 min and addition of 1 M NaOH (280 µL/well), the lysate was neutralized with 5 M HCl prior to the addition of scintillation fluid. The radioactivity of the samples was then quantified by liquid scintillometry (LKB Wallac 1209 Rackbeta, Turku, Finland). Cellular DNA synthesis rate was expressed as incorporation of ^{3}H-thymidine (µCi/mg protein).

2.4.4. Determination of cell migration rates

Cell migration rates were determined by a scratch injury assay. Briefly, the cell monolayers were scratched with a 10 µL pipette tip and were afterwards treated for 24 h with OFE and/or FFE (or the solvent). Images were obtained at 0 and 24 h after the scratch, and quantification was performed using the ImageJ software (NIH, Bethesda, MD, USA).

2.4.5. Protein determination

The protein content of cell monolayers was determined as described by Bradford (1976), using human serum albumin as standard.

2.5. Calculations and statistics

Arithmetic means are given with standard error of the mean (SEM) or standard deviation (SD). The value of n indicates the number of replicates of at least 2 or 3 different replicates.

The extract/drug concentration for 50% inhibition (IC_{50}) was estimated by plotting percentage inhibition with respect to control against treatment concentration *in vitro* tests. $A_{0.5}$ values were determined from absorbance curves at the corresponding wavelength.

Statistical significance of the difference between various groups was evaluated by analysis of variance test followed by the Student-Newman-Keuls test. For comparison between two groups, Student's t test was used. Differences were considered significant when $p < 0.05$.

3. Results and Discussion

3.1. Total polyphenol content (TPC) and flavonoid contents (TFC) of OFE and FFE

The total phenol and flavonoid content of OFE and FFE extracts is shown in Table 1. OFE (174.7 ± 6.0 mg EGA/100 g extract) was proven to have a higher total polyphenol content when compared to FFE (83.2 ± 4.4 mg EGA/100 g extract); similarly, the total flavonoid content of OFE (59.9 ± 2.9 mg EQ/100 g extract) was higher than that of FFE (27.6 ± 1.2 mg EQ/100 g extract). The TPC of OFE reported in the present work (174.7 mg EGA/100 g) agrees with a previous determination of TPC in olive fruits of seven different cultivars of *Olea europaea,* collected in Italy (*Coratina*, *Frantoio*, *Leccino*, *Maiatica*, and *Ogliarola* cultivars) and Algeria (*Sigoise* and *Chemlal* cultivars), which ranged from 147.1 to 290.2 EGA/100 g. However, the TPC was slightly lower than the previously determined in the same olive fruit variety (272.8 EGA/100 g) (Dekdouk et al., 2015). This value is also similar to those observed in the *Chetoui* variety of olive fruits, from Tunisia, which were 313.6, 309.8, 36.1 and 83.3 mg hydroxytyrosol equivalents/100 g during the extraction with hexane, dichloromethane, ethyl acetate and methanol, respectively, although a different standard (hydroxytyrosol equivalents) was used (Brahmi et al., 2015). This variation in TPC may be related to the solvent system used (Brahmi et al., 2015) and the varieties of olive fruits (Dekdouk et al., 2015).

In the case of figs, the TPC content was 83.2 mg EGA/100 g extract, which is lower than that reported by Khadhraoui et al. (2019) for dark dried fig of *Saoudi Douiret* cultivar, but similar to that of green dried fig of *Bayoudhi Douiret* cultivar from Tunisia (TPC of 201.8 and 73.7 mg EGA/100 g, respectively). This value is also lower than those reported for dark colored (*Aberkane*, *Azandjar* and *Bouankik* varieties) and light colored (*Abiarous*, *Azegzaw*, *El-bakour*, *Taamriwth*, *Tahyounte* and *Taghanimt* varieties) dried figs from northern Algeria: the median for dark and light varieties of figs were 619 and 515 mg EGA/100g, respectively (Bachir Bey and Louaileche, 2015). Moreover, they are also lower than the TPC estimated for Iranian (1120-2682 mg EGA/100 g; Pourghayoumi et al., 2017), Indian (331.9 mg EGA/100 g; Vijaya Kumar Reddy et al. (2010) and Turkish (169.4 mg EGA/100 g; dried figs (Capanoglu, 2014). These differences in TPC could be due to many parameters such as the geographical origin, varieties, extraction conditions, and postharvest storage conditions (Bachir Bey et al., 2013; Hoxha et al., 2015).

Flavonoids, a class of polyphenolic secondary metabolites, are one of the most diverse and widespread groups of phytochemicals. These compounds constitute a potential therapeutic alternative for various diseases (Mutha et al., 2021). We verified that the TFC of OFE and FFE were 59.9 ± 2.9 and 27.6 ± 1.2 mg EQ/100 g, respectively. The flavonoid content of olive and Fig fruits are well documented in the literature. The TFC values of OFE obtained is lower than that previously reported for olive extracts of the *Chetoui* variety, from Tunisia, which were 844.6 and 131.2 mg catechin equivalents (EC)/100 g, by dichloromethane and hexane extraction, respectively (Brahmi et al. 2015). Similarly, the TFC of FFE is also slightly lower than those obtained by Bachir Bey and Louaileche (2015), which indicated TFC of dark and light dried fig varieties of 126.6 mg EQ/100 g and 87.2 mg EQ/100 g, respectively. Khadhraoui et al. (2019) reported that the TFC of dried fig extracts from South-Eastern and Middle Eastern Tunisia ranged from 58.0 mg to 112.3 mg EQ/100 g. The variation of TPC could be due to differences in Fig varieties/genotype (Caliskan and Polat, 2012), drying methods (Khadhraoui et al., 2019), solvent used and could also be related to different unit used (catechin or quercetin equivalents).

3.2. Antioxidant activity of OFE and FFE

The antioxidant activity of OFE and FFE was determined using different *in vitro* methods: $DPPH^{\bullet}$ free radical scavenging assay, $ABTS^{\bullet+}$ scavenging assay, CUPRAC assay and the Reducing Power assay. The results of all the tests, expressed in terms of IC_{50} (µg/mL) or $A_{0.5}$, are presented in Table 2. The effectiveness of antioxidant properties is inversely correlated with their $A_{0.5}$ and IC_{50} values.

$DPPH^{\bullet}$ or $ABTS^{\bullet+}$ can accept an electron or a hydrogen atom from the antioxidant compound and will thus be converted to a more stable molecule; this reduction is observed as a color switch for $DPPH^{\bullet}$ (from purple to yellow) and as a decolorization for the radical $ABTS^{\bullet+}$ (Re et al., 1999; Magalhães et al., 2006). Table 2 showed that OFE was more effective in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging assays (IC_{50} value of 29.6 ± 0.8 and 32.4 ± 0.5 µg/mL, respectively) than FFE (IC_{50} > 100 µg/mL in both assays).

In the Reducing power assay, the presence of reductants converts the oxidized form of iron (Fe^{3+}) in ferric chloride to ferrous (Fe^{2+}), which can be monitored by absorbance measurement at 700 nm (Oyaizu, 1986). The CUPRAC assay is based on the reduction of the neocuproine-copper complex, resulting in the formation of a chromogenic complex of copper-neocuproine (Cu(II)-Nc), which absorbs at 450 nm (Saci et al., 2020). The antioxidant capacity of the extracts evaluated with the CUPRAC and the Reducing power assays is presented as $A_{0.5}$ values, which were determined from absorbance curves at the corresponding wavelength (700 and 450 nm, respectively). This reduction was found to be concentration-dependent (results not shown). OFE exhibited a strong ability to reduce iron ($A_{0.5}$ value: 129.8 ± 3.7 µg/mL) and a weaker ability to reduce copper ($A_{0.5}$ value: 29.8 ± 0.9 µg/mL). FFE showed a lower capacity to reduce both iron and copper ($A_{0.5}$ values were > 200 µg/mL and > 100 µg/mL, respectively) (Table 2).

As expected, BHA and BHT, the standard references for the $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and CUPRAC assays, showed a strong scavenger and reducing ability. Ascorbic acid and tannic acids, which were used as standards for the Reducing Power assay, showed also strong reducing activities (Table 2).

Due to the variable responses exerted by a specific antioxidant in various testing systems, it is important to use diverse antioxidant methods to take in account the mechanism of action of each compound (Moukette et al., 2015). The potent antioxidant activity of polyphenols and flavonoids resumes in their ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kasote et al., 2015). The methanolic extract conclusion,

Table 1

Total phenolic content (TPC) and total flavonoid content (TFC) of *Olea europaea* (OFE) and *Ficus carica* (FFE) fruit extracts. Total phenolics content is expressed as mg of gallic acid equivalents per 100 mg of extract (mg EGA/100 mg) and total flavonoid content as mg quercetin equivalents per 100 mg of extract (mg EQ/100 g)[a].

Extract	TPC (mg EGA/ 100 g extract)	TFC (mg EQ/100 g extract)
OFE	174.74 ± 6.03	59.88 ± 2.91
FFE	83.19 ± 4.42	27.64 ± 1.22

[a] Results are expressed as arithmetic means ± SD of three measurements.

Table 2

Antioxidant activity of *Olea europaea* (OFE) and *Ficus carica* (FFE) fruit extracts. Results are expressed as IC_{50} (µg/mL) ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays) or $A_{0.5}$ (µg/mL) (CUPRAC and reducing power assays).

Sample	$DPPH^{\bullet}$ (IC_{50})	$ABTS^{\bullet+}$ (IC_{50})	CUPRAC ($A_{0.5}$)	Reducing Power ($A_{0.5}$)
OFE	29.55 ± 0.77	32.40 ± 0.45	29.83 ± 0.91	129.84 ± 3.72
FFE	>100	>100	>100	>200
BHT	22.32 ± 1.19	1.29 ± 0.30	9.62 ± 0.87	nt
BHA	5.73 ± 0.41	1.81 ± 0.10	3.64 ± 0.19	nt
Ascorbic acid	nt	nt	nt	6.77 ± 1.15
Tannic acid	nt	nt	nt	5.39 ± 0.91

The $A_{0.5}$ (Reducing power and CUPRAC assays) and the IC_{50} values ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays) were calculated by linear regression analysis. Results are expressed as arithmetic means ± SD of three measurements. BHA: Butylated hydroxyl anisole, BHT: Butylhydroxytoluene. nt: Not tested.

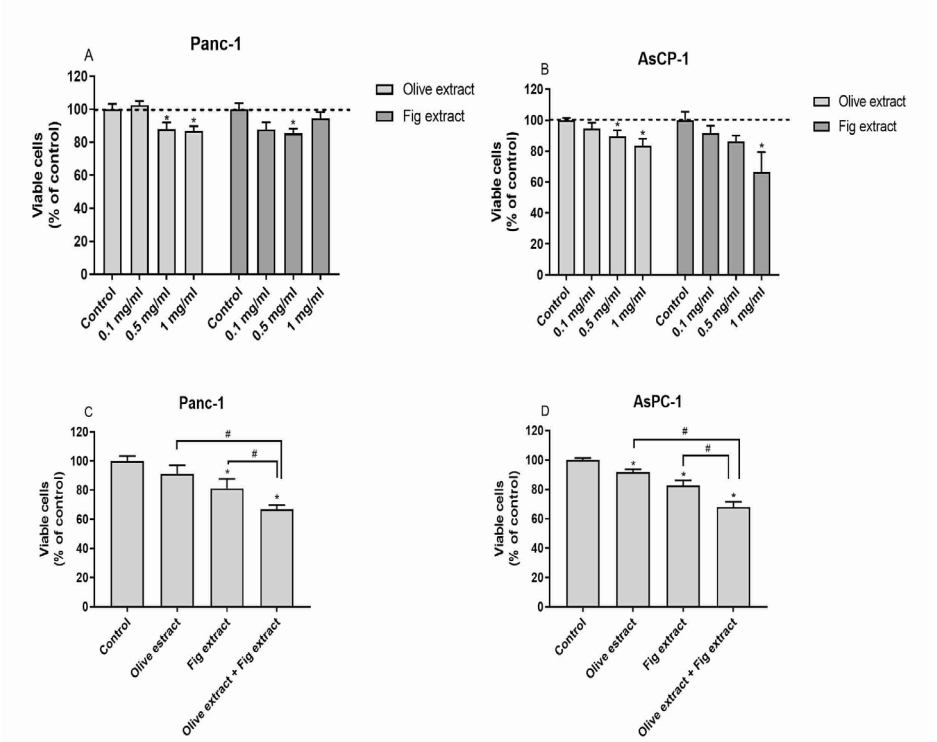

Fig. 1. Effects of *Olea europaea* (OFE) and/or *Ficus carica* (FFE) fruit extracts (24 h) on the viability of PANC-1 and AsPC-1 cells. (A-B) Effect of distinct concentrations of OFE and FFE (n=6); (C-D) Effect of the combination of OFE (0.5 mg/mL) and FFE (0.5 mg/mL) (n=8). Results are presented as arithmetic means ± SEM of two measurements. (A-B) * $p < 0.05$ (Student's t test); (C-D) * $p < 0.05$ *vs* control and $^{\#}$ $p < 0.05$ *vs* each other (ANOVA followed by Student-Newman-Keuls test).

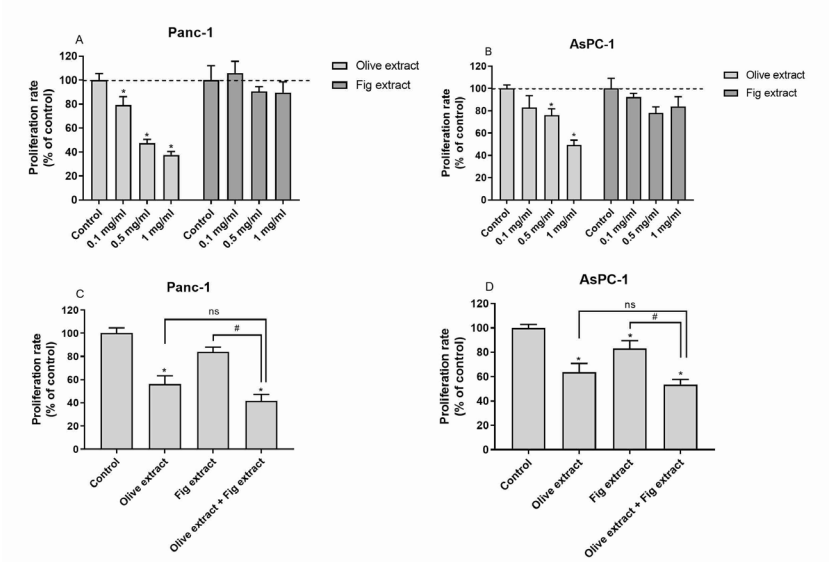

Fig. 2. Effects of *Olea europaea* (OFE) and/or *Ficus carica* (FFE) fruit extracts (24 h) on proliferation of PANC-1 and AsPC-1 cells. (A-B) Effect of distinct concentrations of OFE and FFE (n = 4); (C-D) Effect of combination of OFE (0.5 mg/mL) and FFE (0.5 mg/mL) (n = 6). Results are presented as arithmetic means ± SEM of two measurements. (A-B) *$p < 0.05$ (Student's t test); (C-D) * $p < 0.05$ *vs* control and $^{\#}p < 0.05$ *vs* each other (ANOVA followed by Student-Newman-Keuls test).

of olive fruit (OFE) was found to exhibit a more potent activity as a scavenger of free radicals and as a reducing agent than Fig extract (FFE). Olive fruits are known to have a potent antioxidant activity (Brahmi et al., 2015; Dekdouk et al., 2015), and the major contributors to their antioxidant activity appear to be hydroxytyrosol, tyrosol, *p*-hydroxybenzoic acid, and verbascoside (Dekdouk et al., 2015). So, the more potent antioxidant activity of OFE is most probably correlated with its higher phenolic content (Dekdouk et al., 2015; Brahmi et al., 2015).

3.3. Anticancer activity

3.3.1. Effects of OFE and/or FFE on cell viability

As shown in Fig. 1, OFE and FFE showed promising cytotoxicity against PANC-1 and AsPC-1 cells (Fig. 1A-B). Combination of the extracts clearly increase the cytotoxic effect against both pancreatic cell lines (Fig. 1C-D).

3.3.2. Effect of OFE and/or FFE on cell proliferation

OFE exhibited a marked concentration-dependent antiproliferative effect in both cell lines. In contrast, FFE caused no significant changes in proliferation rates (Fig. 2A-B). When the two extracts were combined, the inhibitory effect was equal to that caused by OFE, in both cell lines (Fig. 2C-D).

A general consensus exists on the importance of dietary habits, including the quantity and quality of food ingested, and their impact on health. Epidemiological studies have clearly established a protective effect of the Mediterranean diet against several types of cancer (Schwingshackl and Hoffmann, 2014; Saha et al., 2017; Mentella et al., 2019; Guasch-Ferré and Willett, 2021), including colorectal (Bamia et al., 2013), gastric (Buckland et al., 2010) and breast (Buckland et al., 2013) cancers.

A positive relationship between olive oil consumption and a reduction in cancer risk has been reported (Romani et al., 2019; Markellos et al., 2022). In agreement with this, our study revealed that OFE possesses a concentration-dependent cytotoxic and antiproliferative effect in both AsPC-1 and PANC-1 pancreatic cancer cell lines. These findings agree with previous reports showing a cytotoxic and/or antiproliferative effect of OFE against other cancer lines, namely hepatic (HepG2) and colorectal (Caco-2) (Maalej et al., 2017; Celano et al., 2019) and a protective effect in a rat model of hepatocellular carcinoma (Shirazi et al., 2018). Moreover, an antiproliferative effect of extracts from *Corregiola* and *Frantoio varieties* of *Olea europaea* L. leaves were reported in the MIA PaCa-2 pancreatic cancer cell line (Goldsmith et al., 2015). The underlying mechanisms by which olive oil exerts a cancer preventive effect have been attributed to its content in various bioactive natural products, specifically polyphenols. Indeed, polyphenols are known to possess several anticancer mechanisms, namely antioxidant, anti-inflammatory, anti-mutagenic, anti-proliferative, pro-apoptotic and anti-angiogenic

(Gorzynik-Debicka et al., 2018). In support of this the main phenolic compounds present in olive extracts (tyrosol, hydroxytyrosol, oleocanthal, oleuropein, rutin, quercetin and glucoside forms of luteolin) have been shown to have anticancer effects on several types of cancers. For instance, hydroxytyrosol induces apoptosis of the LS180 human colorectal carcinoma cell line (Hormozi et al., 2020). Additionally, oleocanthal has antiproliferative and cell cycle inhibitory effects in hepatocarcinoma cells *in vitro* and suppresses tumor growth in an orthotopic hepatocarcinoma model (Pei et al., 2016). It also inhibits invasiveness and tumor growth in breast cancer models (Akl et al., 2014). As to oleuropein, it decreases the number of breast cancer MCF-7 cells by inhibiting the rate of proliferation and inducing cell apoptosis (Han et al., 2009). Importantly, oleocanthal was reported to induce death of the BxPC-3 pancreatic cancer cell line (LeGendre et al., 2015) and Goldsmith et al. (2018) reported a potential antitumoral effect of oleuropein and hydroxytyrosol through reduction of proliferation, influence of cell cycle and induction of apoptosis in MIA PaCa-2 pancreatic cancer cells. However, these compounds showed no effect on other pancreatic cancer cell lines (BxPC-3 and CFPAC-1) (Goldsmith et al., 2018).

Our current study evidenced that FFE possesses a cytotoxic effect against both AsPC-1 and PANC-1 pancreatic cancer cell lines, although no significant antiproliferative effect was verified. The methanolic extract of *Ficus carica* leaves and fruits was previously shown to possess cytotoxic, pronecrotic and proapoptotic effects in Huh7it cancer liver cells (Purnamasari et al., 2019). Very interestingly, a recent study also showed that a *F. carica* fruit extract reduced viability and effectively suppressed migration, metastasis, invasion, and colony formation of PANC-1 and QGP-1 pancreatic cancer cell lines, by inducing apoptotic cell death and autophagy (Ou et al., 2022). However, in contrast to the present study, antiproliferative effects of fig extracts were observed in other cancer cell types. Namely, *F. carica* leaves extract induced anti-growth effects on triple-negative breast cancer MDA-MB-231 cells by promoting apoptosis and cell-cycle arrest at the S phase (Zhang et al., 2018), an antiproliferative effect of fig tree latex was observed on a stomach cancer cell line (Hashemi et al., 2011), and an antiproliferative and proapoptotic effect of fig leaves, whole fruit and latex extracts was observed on HCT-116 and HT-29 colorectal cancer cell lines (Soltana et al., 2019).

A synergic effect has been widely investigated between polyphenols and therapeutical drugs. For instance, quercetin promoted cisplatin-induced apoptosis *in vitro* in human oral squamous cell carcinoma cell lines by downregulating NF-κB and promoted the apoptotic effect of cisplatin *in vivo* in a mouse xenograph model (Li et al., 2019). Moreover, this same combination had a synergic inhibitory effect on cervical cancer cells via inhibition of proliferation, migration, invasion, and stimulation of apoptosis (Xu et al., 2021). In the current study, we were interested in verifying the effect of combination of the two extracts against AsPC-1 and PANC-1 cell lines. Our findings show a potent additive cytotoxic and antiproliferative effect of combination of OFE and FFE against these two cell lines. This result may be explained by the additive effect of phenolic compounds present in the two extracts, as previously reported when combining quercetin and sulforaphane (Srivastava et al., 2011), isoflavone and curcumin (Wang et al., 2008) and ferulic acid and δ-tocotrienol (Eitsuka et al., 2014).

3.3.3. Effect of OFE and/or FFE on cell migration

As shown in Fig. 3, OFE evidenced a concentration-dependent antimigratory effect in PANC-1 cells, but no effect on AsPC-1 migration rates was found. As for FFE, it exhibited a marked and concentration-dependent stimulatory effect on the migration rates of both cell lines (Fig. 3A-B). More interestingly, combination of the two extracts led to a complete abolition of the stimulatory effect of FFE in the two cell lines; instead, an effect very similar to that caused by OFE was observed (Fig. 3C-D).

Directional cell migration plays a key role in pivotal steps that promote tumor metastasis, such as cellular migration and invasion into the surrounding stroma. Given the fact that current chemotherapy drugs for pancreatic cancer only offer an increase in survival of up to six months (Moore et al., 2007; Park et al., 2021), the search for novel agents with an antimigratory effect on pancreatic cancer cells while displaying limited toxicity towards normal cells is thus paramount. The present data clearly demonstrate that:

(1) OFE showed a concentration-dependent antimigratory effect towards PANC-1 cells only.

(2) OFE presented a marked stimulatory effect on the migration of both cell lines.

(3) Very interestingly, combination of the two extracts resulted in a complete abolition of the stimulatory effect of FFE and instead an effect comparable to that caused by OFE was found. Although we have no evidence as to the mechanism involved in this effect of OFE, components in OFE appear to be able to abolish the stimulatory effect of FFE on cell migration.

Several studies have tested the ability of crude extracts rich in phenolic compounds or isolated phenolic compounds in inhibiting migration of pancreatic cancer cells *in vitro* and *in vivo*. Fahrioğlu et al. (2016) reported that ferulic acid exhibits an anticancer effect by inhibiting migration of MIA PaCa-2 pancreatic cancer cells. Similarly, kaempferol effectively inhibited the migratory activity of MIA PaCa-2, PANC-1, and SNU-213 human pancreatic cancer cell lines at relatively low concentrations (Lee and Kim, 2016). From our findings, we believe that the use of OFE, either alone or in combination with pro-migratory agents, can inhibit pancreatic cell migration, which is very interesting.

4. Concluding remarks

Collectively, this report demonstrates that OFE, which is very rich in bioactive polyphenolic compounds, possesses anticancer effects against PANC-1 and AsPC-1 pancreatic cancer cell lines. It exhibits cytotoxic, antiproliferative and antimigratory effects. In contrast, FFE exhibits a cytotoxic effect in these two cell lines,

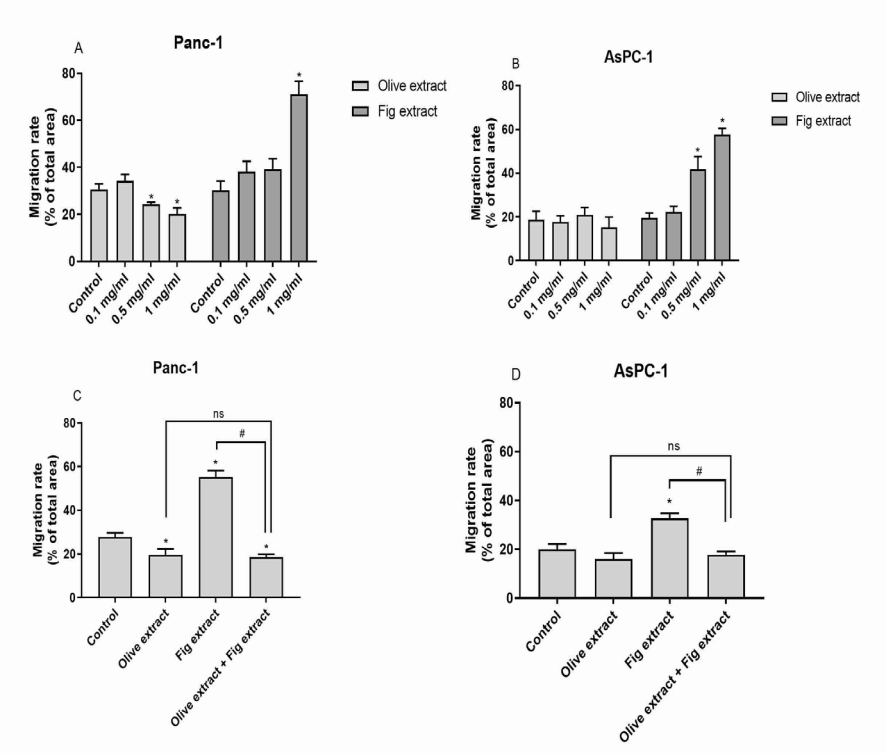

Fig. 3. Effects of *Olea europaea* (OFE) and/or *Ficus carica* (FFE) fruit extracts (24 h) on the migration capacity of PANC-1 and AsPC-1 cells. (A-B) Effect of distinct concentrations of OFE and FFE (n = 6); (C-D) Effect of combination of OFE (0.5 mg/mL) and FFE (0.5 mg/mL) (n=8). Results are presented as arithmetic means ± SEM of two measurements. (A-B) *$p < 0.05$ (Student's t test); (C-D) *$p < 0.05$ *vs* control and $^{\#}$ $p < 0.05$ *vs* each other (ANOVA followed by Student-Newman-Keuls test).

but it does not have an antiproliferative effect and even stimulates the migration of these cells. The combination of OFE and FFE led to an increase in the cytotoxic effect in both cell lines and the disappearance of the promigratory effect of FFE. Thus, the combination of these extracts in fact exerts a more interesting anti-cancer effect than either of the extracts alone.

This report also demonstrates that OFE and FFE exhibit different antioxidant properties in response to different models, namely DPPH$^{\bullet}$, ABTS$^{\bullet+}$, CUPRAC and Reducing Power models, which may be related to their different polyphenol and flavonoid content. More importantly, the higher phenolic and flavonoid content and antioxidant ability of OFE, in relation to FFE, correlates with a more prominent anticancer effect. Because the biological activity of polyphenols is strongly related to their antioxidant properties (Gorzynik-Debicka et al., 2018), we suggest that the anticancer effect of the extracts results from their antioxidant properties.

This study could lead to new research on pancreatic cancer and the consumption of olive and fig products, and place OFE and FFE/ phenolic compounds as good candidates for further molecular studies aimed at finding a better, and more effective treatment approach for resistant pancreatic cancer. In future work, it would be very interesting to confirm the results of this study in other human pancreatic cancer cell lines, and also in animal pre-clinical models. Moreover, the mechanisms involved in the positive interaction between OFE and FFE should be further explored.

List of abbreviations

ABTS: 2,2-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid; **AsPC-1 Cell Line**: Human Pancreatic Ascites Adenocarcinoma Cell Line; **ATCC**: American Type Culture Collection; **BHA**: 2,6-Di-*tert*-butyl-4-hydroxy toluene; **BHT**: Butylated Hydroxyltoluene; **CUPRAC**: Cupric Acid Reducing Antioxidant Capacity; **DMSO**: Dimethylsulfoxide; **DPPH$^{\bullet}$**: (1,1-Diphenyl-2-picrylhydrazil) Scavenging Assay; **FFE**: *Ficus carica* Extract; HEPES: *N*-2-Hydroxyethylpiperazine-*N'*-2-ethanesulfonic Acid; **MTT**: 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; OFE: *Olea europaea* Extract; **PANC-1 Cell Line**: Human Pancreatic Epithelioid Carcinoma Cell Line; **SD**: Standard Deviation; **SEM**: Standard Error of the Mean; **TFC**: Total Flavonoid Content; **TPC**: Total Phenolic Content.

Author contribution statement

All authors contributed to the study's conception and

design. Nadia Dekdouk, Souad Ameddah, Chawki Bensouici, Meghboun Ibtissem and Menad Ahmed performed material preparation, experiments, data collection, and analysis. Nadia Dekdouk and Fátima Martel wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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