

# Chemical Composition, Antioxidant Potential, and Anticancer Activity of *Origanum compactum* Benth Extract Against Human Lung and Pancreatic Cancer Cells

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Received: 23.10.2024; Accepted: 24.02.2026; Published: 30.03.2026

**Abstract:** Cancer remains a major global health challenge, with current treatments often limited by resistance, toxicity, and variable effectiveness. In the search for improved therapies, medicinal and aromatic plants represent a valuable source of bioactive compounds with remarkable therapeutic potential. These naturally derived compounds play a pivotal role in the prevention and treatment of various diseases, including cancer, offering multi-targeted actions with lower toxicity and potential to enhance conventional treatments. This makes them promising candidates for the development of novel cancer therapies. In this study, the ethanolic extract of *Origanum compactum*, obtained by accelerated solvent extraction (ASE300), was evaluated for its phenolic content, antioxidant activity, and cytotoxicity against Human Lung (NCI-N417) and Pancreatic (TCP-1026) cancer cell lines. GC-MS analysis identified twenty-two compounds in the *O. compactum*, with  $\gamma$ -terpinene, thymol, and carvacrol as the dominant compounds. The extract is rich in phenolic compounds and showed a potent antioxidant activity with a DPPH-radical-scavenging activity of  $IC_{50}=31.89 \pm 0.05 \mu\text{g mL}^{-1}$ . *O. compactum* extract also exhibits a noticeable antiproliferative potential against Human Lung ( $IC_{50}=304.8 \pm 0.18 \mu\text{g mL}^{-1}$ ) and Pancreatic cancer cells ( $IC_{50}=58.87 \pm 0.04 \mu\text{g mL}^{-1}$ ). These findings suggest that *O. compactum* possesses significant antioxidant and anticancer properties, highlighting its potential as a source of bioactive compounds for cancer therapy. However, further studies, including *in vivo* investigations and the mechanism of action, are necessary to confirm its efficacy and safety for clinical applications.

**Keywords:** *Origanum compactum*; antioxidant; anticancer; lung cancer; pancreatic cancer.

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## 1. Introduction

Cancer represents a leading cause of death worldwide. According to the International Agency for Research on Cancer (IARC), the global cancer burden increased to 19.3 million new cases and nearly 10 million deaths in 2020. Despite advancements in the treatment of cancer, several available anticancer drugs are associated with drug resistance and serious side

effects [1–3]. Therefore, the development of new anticancer agents remains a current challenge. Medicinal plants have played a pivotal role in drug development. Herbal medicine is still widely used, particularly in developing countries, due to either the unavailability or high cost of many contemporary pharmaceutical medications [4]. Moreover, plant-derived products have gained increasing popularity worldwide, as they are commonly perceived as safe by consumers.

Several studies have revealed that regular consumption of vegetables, fruits, and many medicinal plants helps reduce the risk of cancer. Indeed, it has been shown that phytochemicals from medicinal plants possess anticancer properties, including antioxidant, anti-inflammatory, and pro-apoptotic activities [5–7]. Currently, some plant-derived compounds are clinically used in cancer chemotherapy, such as vincristine (an alkaloid extracted from *Catharanthus roseus*), which has been approved by the US Food and Drug Administration (FDA) to treat acute lymphocytic leukemia, lymphoid blast crisis of chronic myeloid leukemia, and Hodgkin and Non-Hodgkin lymphoma [8].

Morocco is recognized for its rich botanical diversity in the Mediterranean region, with about 4,200 species [9]. Medicinal plants are traditionally used by the Moroccan population to manage numerous human diseases, including cancers. Studies reported that among 103 plant species traditionally used to treat cancer in Morocco, the dominant botanical family was Lamiaceae, with 11 species, including *Origanum compactum* [10,11]. *Origanum compactum* Benth. is an endemic species to Morocco and southern Spain. This perennial herb is distinguished by its pubescent and large oval leaves, villous stems, larger bracts, and compact inflorescence [12]. It is known in Morocco as “Zaâtar”, and is considered among the most popular and appreciated medicinal plants used in traditional medicine and as a culinary herb. Ethnobotanical studies have reported that the leaves and flowering tops of *O. compactum* are widely used either in mixtures or separately for the treatment of many diseases [13,14]. In addition, *O. compactum* extracts have shown diverse and potent biological activities such as antibacterial [15], insecticidal [16], antioxidant [17], antiinflammatory [18], antimutagenic [19], and anticancer [18,20,21] activities.

This study aimed to determine the chemical composition, polyphenol content, and antioxidant and antiproliferative activities of an *O. compactum* ethanolic extract obtained via accelerated solvent extraction (ASE). The antiproliferative activity was evaluated against two cancer cell lines: small cell bronchopulmonary carcinoma (SCLC) (NCI-N417) and a Pancreatic Cancer cell line (TCP-1026) using the MTT test.

## 2. Materials and Methods

### 2.1. Plant material.

The leaves of *Origanum compactum* Benth. were collected from the province of Tetouan (northern Morocco). The plant was identified at the Plant Biology Laboratory, Faculty of Sciences, Abdelmalek Essaâdi University, and a specimen (FS-LB-1701) was deposited.

### 2.2. Pressurized liquid extraction.

The leaves of *O. compactum* were shade-dried at room temperature for a week and finely ground in a mechanical grinder (planetary ball mill with stainless steel buckets). The extraction was then carried out using maceration and pressurized liquid extraction (PLE) according to the protocol described by Amkiss *et al.* [22].

The ground material (10 g) was macerated in one liter of ethanol for 24 h with magnetic stirring at room temperature. The samples were centrifuged for 10 min (4,000 rpm, 4°C) to collect the supernatant. The remaining residue was then re-extracted under the same conditions (exhaustion of the plant material). The recovered solution was first filtered, under vacuum, through a grade filter (Whatman paper) to remove large particles, then through a Millipore Millex-HV hydrophilic filter (0.45 and 0.25 µm, respectively). The filtrates were subsequently concentrated using a rotary evaporator with a vacuum pump (Rotavapor R-215 with Vacuum Pump V-710, Büchi, Switzerland) and stored at -20°C until further use.

The pressurized liquid extraction (PLE) was performed using a Dionex ASE-300 Accelerated Solvent Extractor (Voisins le Bretonneux, France). The *O. compactum* powder (5g) was mixed with Fontainebleau sand (70 g). A cellulose filter was placed at the bottom of the 66 mL stainless steel extraction cell, and then, the mixture was added to it. The sample was mixed with the dispersing agent to obtain a homogeneous extraction. The cell was then closed and placed on the ASE carousel for extraction with ethanol solvent. The filtrates were concentrated using a rotary evaporator with a vacuum pump (Rotavapor R-215 with Vacuum Pump V-710, Büchi, Switzerland) and stored at -20°C until further use.

### 2.3. Quantification of phenolic compounds: Folin-Ciocalteu method.

Total phenols were determined using the Folin-Ciocalteu method according to the procedure described by Li *et al.* [23]. Briefly, 2.5 mL of the Folin-Ciocalteu solution (diluted tenfold in distilled water) was added to 0.5 mL of the extract (prepared in the solvent with suitable dilutions) and then homogenized. After 5 min of incubation at 50°C, 2 mL of 7.5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) solution was added to the mixture. The absorbance was measured with a spectrophotometer (Agilent 8453E UV-visible spectroscopy system) at 765 nm against a blank sample. Polyphenols in the extract were expressed as gallic acid equivalents (µg GAE/mg dry extract). The quantification was performed in triplicate.

### 2.4. Chemical composition.

GC/MS analysis of the extract was carried out on a Thermo Finnigan Voyager GC/MS with Trace 2000 GC (Spectralab, Canada) equipped with a 30 m long DB-5 MS (5% phenyl, 95% dimethyl polysiloxane) capillary column with an internal diameter of 0.25 mm and a stationary phase film thickness of 0.25 µm. The furnace temperature gradient ranged from 50°C to 300°C at 5°C min<sup>-1</sup>. The flow rate of the carrier gas helium was set at 1 mL min<sup>-1</sup>. The injected volume was 1 µL. The acquisition of the mass spectrum was performed on a quadrupole mass spectrometer with an m/z range from 40 to 500 amu at 0.1 scan s<sup>-1</sup> and 70 eV electron ionization energy. The identification of the quantified compounds was verified by the presence of both quantitation and confirmation ions, as well as by comparing the observed retention times with those obtained from the calibration standards and the NIST/EPA/NIH Mass Spectral Library (EI) mass spectra.

### 2.5. Antioxidant activity.

The antioxidant effect of *O. compactum* was evaluated using three complementary tests: the β-carotene bleaching test, DPPH radical scavenging activity, and the FRAP method.

### 2.5.1. Anti-radical power (DPPH test).

The DPPH free radical scavenging assay was performed in triplicate according to the method of Blois [24] as described by Brand-Williams *et al.* [25]. Different concentrations (0–250  $\mu\text{g mL}^{-1}$ ) of the plant extract obtained by ASE and reference standards (BHT, ascorbic acid, and Trolox) were tested. Briefly, 3 mL of the diluted extract was added to 1 mL of DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution (0.1 mmol) in methanol. The mixture was placed in the dark at room temperature for 30 min. The hydrogen transfer reaction from the antioxidant to the DPPH was followed by visible spectrophotometry at a wavelength of 517 nm against a corresponding blank. The DPPH-scavenging activity (%) was calculated using the following relationship:

$$\% \text{ Inhibition} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100 \quad (1)$$

The  $\text{IC}_{50}$  value, defined as the concentration of the test sample leading to a 50% reduction in free radical concentration, was calculated as  $\mu\text{g mL}^{-1}$  through a sigmoidal dose-response curve.

### 2.5.2. Reducing power (FRAP method).

The ferric reducing antioxidant power assay was measured according to the procedure described by Oyaizu [26]. Briefly, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of a 1% solution of potassium ferricyanide were added to a series of test tubes containing 1 mL of the extract at different concentrations (50–1,000  $\mu\text{g mL}^{-1}$ ). The mix was heated at 50°C in a water bath for 20 min. A volume of 2.5 mL of trichloroacetic acid (10%) was then added, and the mixture was centrifuged at 3,000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride ( $\text{FeCl}_3$ ) (0.1%). A blank without a sample was prepared under the same conditions. The readings were taken at 700 nm, using BHT as a positive control. Analyses were performed in triplicate.

### 2.5.3. $\beta$ -carotene bleaching assay.

The  $\beta$ -carotene/linoleic acid bleaching assay was performed in triplicate following the method described by Kartal *et al.* [27]. The emulsion of  $\beta$ -carotene/ linoleic acid was prepared by dissolving 0.5 mg of  $\beta$ -carotene in 1 mL of HPLC-grade chloroform, followed by the addition of 25  $\mu\text{L}$  of linoleic acid, and 200 mg of Tween 20 was added. The chloroform was completely evaporated using an evaporating centrifuge (DyNA Vap Centrifugal Evaporator). Subsequently, 100 mL of oxygen-saturated water was then added with vigorous agitation. From this mixture, 2.5 mL was transferred to separate test tubes containing 350  $\mu\text{L}$  of the tested extract (2 g  $\text{L}^{-1}$ ) or reference antioxidant (BHT). Absorbance was measured for BHT at 490 nm. The oxidation of the  $\beta$ -carotene emulsion was monitored by measuring the absorbance at 490 nm at regular time intervals (every 30 min) for 48 h. The relative antioxidant activity of the extracts (RAA) was calculated as:

$$\% \text{ Inhibition} = \left[ \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right] \times 100 \quad (2)$$

## 2.6. Anticancer activity.

Two human tumor cell lines obtained from the American Type Culture Collection (ATCC), the NCI-N417 cell line, a type of small cell bronchopulmonary carcinoma (SCLC), and the TCP-1026 cell line of the Pancreatic Cancer Panel were used for cytotoxic assay. The MTT cytotoxicity was performed according to the method previously described by Liu *et al.* [28]. Cells cultured in RPMI-1640 medium supplemented with 10% FCS were inoculated into wells of a 96-well plate at a density of  $14.103 \text{ cells mL}^{-1}$  ( $100 \mu\text{L/well}$ ). After 24 h of culture, the cells were placed in the presence or not of increasing concentrations (from 0.039 mg to 5 mg performed in sextuplicate) of the *O. compactum* extract. The plates prepared in triplicate were incubated for 72 h at  $37^\circ\text{C}$  with a 5%  $\text{CO}_2$ , 95% air, and complete humidity. Then,  $20 \mu\text{L}$  of MTT solution ( $5 \text{ mg mL}^{-1}$ , in PBS buffer) (Sigma, Madrid, Spain) was added to each well. Then, the plates were incubated at  $37^\circ\text{C}$  for 3–4h in a humidified atmosphere and 5%  $\text{CO}_2$  (this step is necessary for MTT to enter the cells either by endocytosis or via a membrane transporter) [28]. The cells were then centrifuged (600 rpm) for 10 min, and the supernatant was carefully aspirated using a multi-channel vacuum suction tip. Then,  $150 \mu\text{L}$  of DMSO was added to each well to dissolve the formazan crystals [29]. The plates were subsequently shaken on a rotating plate for 10 min and placed in an ELISA plate reader (Dynatech MP 7000) to measure the optical density (550 nm) and estimate the number of living cells in each well. The activity was performed in triplicate, and results were expressed as percent survival relative to untreated cells (control cells) as:

$$\text{Cell growth (\%)} = [A (\text{sample})/A (\text{control})] \quad (3)$$

The concentration that induces 50% growth inhibition ( $\text{IC}_{50}$ ) was determined by non-linear regression analysis with the equation of a sigmoidal dose–response curve (Graph Pad Prism 6).

## 2.7. Statistical analysis.

All experiments were performed in triplicate. The data were expressed as mean  $\pm$  SD and analyzed by the one-way ANOVA, followed by the Tukey test ( $p < 0.05$ ), using GraphPad Prism V 6.00.

## 3. Results and Discussion

The extraction yield of *O. compactum* using ASE is approximately 17.5%. The chemical composition, polyphenol content, and the antioxidant and anticancer activities of the crude extract were investigated.

### 3.1. Chemical composition.

The analysis of the chemical composition of *O. compactum* ethanolic extract by GC/MS identified 22 compounds. The chemical profile of the extract is dominated by  $\gamma$ -terpinene (33.34%), thymol (13.34%), carvacrol (8%), and germacrene-D (7.66%) (Table 1).

**Table 1.** Chemical composition of *O. compactum* ethanolic extract.

	Components	RT	%
1	$\alpha$ -Thujene	4.99	0.34
2	$\alpha$ -Pinene	5.05	0.32
3	Camphene	5.65	0.34
4	Sabinene	5.79	0.36
5	$\beta$ -Pinene	6.01	0.37
6	Myrcene	6.42	0.3
7	$\alpha$ -Phellandrene	6.86	0.26
8	$\alpha$ -Terpinene	8.21	2
9	<i>p</i> -Cymene	9.19	3
10	$\gamma$ - Terpinene	9.28	33.34
11	trans sabinène hydrate	10.2	2.67
12	Borneol	12.69	1.73
13	2-isopropyl-1-méthoxy-4-méthylbenzene	13.23	2
14	<i>p</i> -Mentha-3.6-diene-2.5-dione	14.57	2.87
15	Thymol	15.26	13.34
16	Carvacrol	15.57	8
17	carvacryl acétate	15.97	2.66
18	Caryophyllene	19.2	1.34
19	Germacrene-D	20.15	7.66
20	Caryophyllene oxyde	24.14	5.67
21	Tricosane	35.56	1
22	Pentacosane	39.33	0.66

According to the literature, the *O. compactum* extracts contain monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpene hydrocarbon compounds, where carvacrol, thymol, *p*-cymene,  $\gamma$ -terpinene, carvacryl methyl ether, and  $\alpha$ -terpineol are dominant constituents that are known for their potent pharmacological properties [11]. The chemical composition of *O. compactum* extracts can be influenced by plant genetics and geographical origin, harvesting date, plant part used, and extraction method [11,16].

### 3.2. Total polyphenol content.

The quantification of total phenolic compounds in the ethanolic extract obtained by ASE revealed that *O. compactum* is particularly rich in these compounds, with a content of  $285.18 \pm 21.47 \mu\text{g GAE mg}^{-1}$  extract (Table 2). Phenolic compounds include phenolic acids, flavonoids, and tannins. The total phenolic content reported in this investigation is significantly higher than that of the ethanolic extract of both *O. compactum* ( $106.9 \mu\text{g GAE mg}^{-1}$ ) [30] and *O. vulgare* ( $55.35 \mu\text{g GAE mg}^{-1}$ ) [31]. This could be attributed to the ASE extraction method used, a method that is considered an environmentally friendly approach that can significantly reduce extraction time and solvent consumption, and enhance both the yield and quality of the extract [32].

**Table 2.** Total polyphenol content and antioxidant activity of *O. compactum* extract in comparison to reference standards (BHT, ascorbic acid, and Trolox).

	Total phenol contents ( $\mu\text{g GAE mg}^{-1}$ )	DPPH (IC <sub>50</sub> ) ( $\mu\text{g /mL}$ )	Reducing power (EC <sub>50</sub> ) DO <sub>700nm</sub>	$\beta$ -carotene (%)
<i>O. compactum</i>	$285.18 \pm 21.47$	$31.89^c \pm 0.05$	$1.367^b \pm 0.00$	$100^a \pm 0.013$
BHT	-	$41.16^b \pm 0.05$	$1.489^a \pm 0.063$	$97.93^b \pm 0.016$
Ascorbic acid	-	$69.99^a \pm 0.046$	-	-
TROLOX	-	$22.53^d \pm 0.11$	-	-

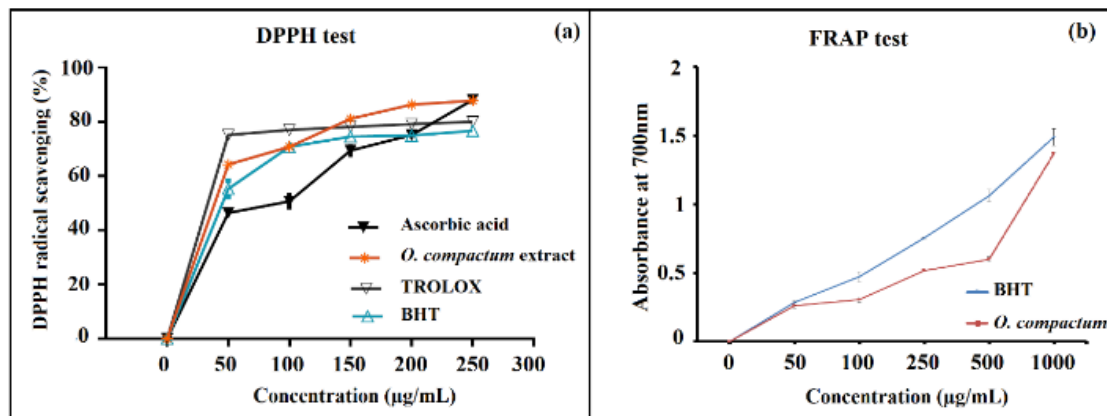
### 3.3. Antioxidant activity.

Oxidative stress, produced by reactive oxygen species (ROS), can damage DNA and proteins in cells, leading to the development of many diseases, such as cancer. Natural compounds with high antioxidant activity play a key role in free radical scavenging and

promoting DNA damage repair [33]. *Origanum compactum* extract showed significant antioxidant activity, with high DPPH radical-scavenging power and a noticeable capacity to reduce ferric ions and to couple the oxidation of linoleic acid and  $\beta$ -carotene (Table 2).

### 3.3.1. DPPH scavenging effect.

The ethanolic extract of *O. compactum* showed strong DPPH-radical scavenging activity in a dose-dependent manner, with an  $IC_{50}$  value of  $31.89 \pm 0.05 \mu\text{g mL}^{-1}$  (Table 2). Its antioxidant activity was significantly higher than that of BHT ( $41.16 \pm 0.05 \mu\text{g mL}^{-1}$ ) and ascorbic acid ( $67 \pm 0.04 \mu\text{g mL}^{-1}$ ), but slightly lower than that of Trolox ( $22.53 \pm 0.11 \mu\text{g mL}^{-1}$ ). The scavenging effect increased progressively with rising extract concentration (Figure 1a).



**Figure 1.** (a) DPPH radical-scavenging activity; (b) reducing power of *O. compactum* extract compared to reference standards. Each value represents the mean of three measurements  $\pm$  standard deviation.

### 3.3.2. Reducing power.

The ethanolic extract of *O. compactum* showed a potent ferric ion-reducing power in a concentration-dependent manner. The reducing activity increased progressively with rising extract concentration, with a notable enhancement observed from  $50 \mu\text{g mL}^{-1}$  ( $DO_{700\text{nm}} = 0.263 \pm 0.018$ ) and reaching a maximum value of  $DO_{700 \text{ nm}} = 1.367 \pm 0.00$  at  $1,000 \mu\text{g mL}^{-1}$ . This variation in reducing capacity was directly related to extract concentration and was compared to that of BHT (Figure 1b).

### 3.3.3. Inhibition of $\beta$ -carotene oxidation.

Results showed that both BHT and *O. compactum* extract inhibited the coupled oxidation of linoleic acid and  $\beta$ -carotene compared with the negative control, which represented 100% peroxidation. The strong antioxidant activity of *O. compactum* observed in this study is attributed to its chemical composition. Previous studies showed that the dominant compounds of *O. compactum* extract, such as carvacrol, thymol, and p-cymene, possess potent antioxidant properties [34,35]. These findings highlight the health benefits of oregano consumption, supporting its broad applications in the food and pharmaceutical fields [36]. According to the literature, the antioxidant activity of *O. compactum* extracts can vary depending on the extraction method, solvent used, and phenological stage. For example, El Babili *et al.* [30] reported that the aqueous extract possesses higher DPPH-radical scavenging activity than ethyl acetate, ethanol, and petroleum ether extracts. Similarly, another study found significant variability in the anti-radical activity of *O. compactum* essential oil (EO) according

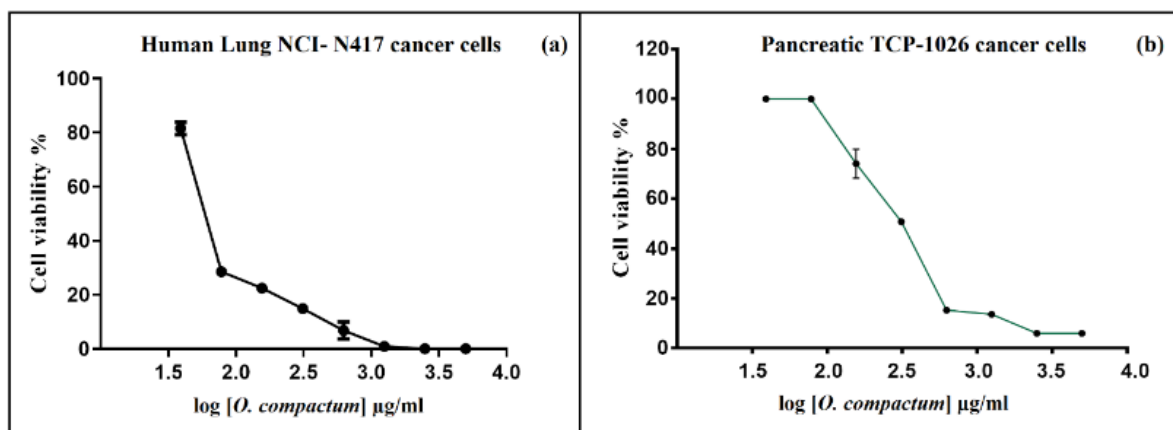
to the phenological stage, with EO from plants collected at the post-flowering stage showing the highest DPPH-radical scavenging and reducing power activities [37].

### 3.4. Antiproliferative activity.

The results of the in vitro antiproliferative assay using the MTT test revealed significant cytotoxicity of the *O. compactum* ethanolic extract against both Lung and Pancreatic cancer cell lines. Lung cancer represents the most common cause of cancer-related mortality in both men and women worldwide, accounting for approximately 1.8 million deaths annually [38]. Pancreatic cancer, on the other hand, is a devastating gastrointestinal cancer known for its late detection, limited treatment efficacy, and poor prognosis [39].

The MTT assay is based on the capacity of mitochondrial dehydrogenase enzyme in viable cells to reduce the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple formazan product, which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cell lines [40].

The results showed that *O. compactum* extract exhibits significant antiproliferative activity against both Human Lung (NCI-N417) and Pancreatic Cancer (TCP-1026) cell lines, with IC<sub>50</sub> values of 304.8±0.18 µg mL<sup>-1</sup> and 58.87±0.04 µg mL<sup>-1</sup>, respectively. These activities varied in a dose-dependent manner, as increasing extract concentrations led to a progressive decrease in living cell numbers (Figure 2). The variation of cell viability as a function of extract concentration responds to a classical sigmoidal dose-response curve when expressed on a semi-logarithmic graph. Figure 2 indicates that the *O. compactum* extract showed a proliferation-inhibitory effect at all concentrations tested against the NCI-N417 and TCP-1026 cell lines, with inhibition percentages of 84.70% and 91%, respectively.



**Figure 2.** Antiproliferative effect of *O. compactum* extract against (a) Human Lung NCI- N417; (b) Pancreatic TCP-1026 cancer cells using MTT test.

The results show that *O. compactum* ethanolic extract is more effective against Pancreatic Cancer (TCP-1026) cells than against Human Lung (NCI-N417) cell line. Lung and pancreatic cancers differ markedly in their biology and clinical behavior. Pancreatic cancer, though less common, is among the most lethal, characterized by an extensive fibrotic stroma that limits drug penetration and contributes to intrinsic chemoresistance. Its molecular profile is dominated by almost universal KRAS mutations along with frequent alterations in TP53, SMAD4, and CDKN2A, factors that underpin its aggressive course and poor prognosis [41].

In contrast, lung cancer, one of the most prevalent malignancies, often develops in a highly vascularized microenvironment and may respond initially to targeted therapies [42].

Previous studies have reported that the anticancer effects of plant extracts and biomolecule compounds may vary depending on the cancer type and, in some cases, even among different cell lines of the same cancer type [43]. For instance, Li *et al.* [44] reported that carvacrol possesses high anticancer activity against the MDA-MB-231 breast cancer cell line, while the MCF-7 breast cancer cell line was less sensitive.

Although few studies have investigated the anticancer properties of *O. compactum*, available ones suggest a broad spectrum of activity. Reported findings include significant anticancer activity against Human breast cancer cells MCF7 [20,30], SMMC-7721 hepatoma cells [45], and epidermoid carcinoma (skin cancer cell line A431) [18], as well as a strong antimutagenic effect against urethane-induced mutagenicity in *Drosophila melanogaster* [19]. Moreover, essential oils of *O. compactum* have cytotoxicity against lung cancer A549 and H460 cell lines (IC<sub>50</sub> value of 109 nL/mL against H460) [21,45], cervical adenocarcinoma (IC<sub>50</sub> value of 73 nL/mL against HeLa), and colorectal carcinoma (HCT116) with an IC<sub>50</sub> value of 154 nL/mL [21]. These results support the health benefits of the regular and rational consumption of *O. compactum*.

Furthermore, the major compounds of *O. compactum* extract, particularly carvacrol [46] and thymol [47], are known for their significant anticancer activities. The cytotoxic effect of carvacrol has been linked to the induction of apoptosis, characterized by increased Bax expression, decreased mitochondrial membrane potential, caspase activation, and PARP cleavage [46]. Additionally, it has been shown to modulate the TRPM7 signaling pathway [44]. However, the anticancer activity of the plant extract could be the result of interactions between many compounds.

In the same *Origanum* species, the anticancer mechanisms of action appear to be diverse. The essential oil of *O. vulgare* subsp. *hirtum* exhibits significant anticancer activity via immune modulation and immunity-mediated apoptosis induction [48]. Likewise, potent anticancer and antimetastatic effects of *O. syriacum* have been demonstrated against the aggressive TNBC phenotype by regulating cell adhesion, invasion, migration, and angiogenesis through activation of p38 MAPK pathways and inhibition of STAT3 signaling. Moreover, the ethanol extract of *O. syriacum* could induce cell cycle arrest in MDA-MB-231 cells, suppress angiogenesis, and promote apoptosis [49].

#### 4. Conclusions

The present study underlines the effectiveness of the environmentally friendly PSE extraction techniques in enhancing plant extract yield. *O. compactum* extract was identified as a rich source of phenolic compounds that contain high amounts of  $\gamma$ -terpinene, thymol, and carvacrol, which contribute to its strong antioxidant properties. Moreover, the *in vitro* assays demonstrate a significant anticancer potential against Pancreatic and Lung cancer cell lines, with particular efficacy against Pancreatic cancer cells. These findings emphasize the value of *O. compactum* herbs and their extracts as valuable reservoirs of bioactive compounds, with remarkable biological activities. Moreover, they support the potential role of medicinal and aromatic plants, including *O. compactum*, in cancer prevention and as complementary agents to conventional therapies, owing to their diverse bioactive compounds that may enhance efficacy and reduce side effects. Further in-depth studies, including *in vivo* evaluations and

mechanistic investigations, are necessary to validate the therapeutic relevance of *O. compactum* and its bioactive constituents.

### Author Contributions

Conceptualization and methodology, S.A., M.I., and J.B.L.S.; software, S.A. and M.B.; validation, S.A., B.M., S.B., A.D.R.L., J.B.L.S., and M.I.; formal analysis, S.A., B.M. and S.B.; investigation, S.A., J.B.L.S., A.D.R.L., and M.I.; resources, J.B.L.S. and M.I.; writing—original draft preparation, S.A., M.B., and S.B.; writing—review and editing, S.A., B.M., S.B., A.D.R.L., J.B.L.S., and M.I.; supervision, M.I. All authors must confirm their agreement with the contribution statement before submission.

### Institutional Review Board Statement

Not Applicable.

### Informed Consent Statement

Not Applicable.

### Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

### Funding

This research received no external funding.

### Acknowledgments

Declared none.

### Conflicts of Interest

The authors declare no conflict of interest.

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