



**HAL**  
open science

## Phytochemical analysis, cytotoxic and antioxidant activities of *Myrtus communis* essential oil from Morocco

Younes Harassi, Mounir Tilaoui, Abderrazak Idir, Frédéric Jullien, Sylvie Baudino, Sana Ajouaoui, Hassan Ait Mouse, Abdelmajid Zyad

### ► To cite this version:

Younes Harassi, Mounir Tilaoui, Abderrazak Idir, Frédéric Jullien, Sylvie Baudino, et al.. Phytochemical analysis, cytotoxic and antioxidant activities of *Myrtus communis* essential oil from Morocco. *Journal of Complementary and Integrative Medicine*, 2019, 16 (3), pp.20180100. 10.1515/jcim-2018-0100 . hal-04695467

**HAL Id: hal-04695467**

**<https://hal.science/hal-04695467v1>**

Submitted on 12 Sep 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Younes Harassi<sup>1</sup> / Mounir Tilaoui<sup>1</sup> / Abderrazak Idir<sup>1</sup> / Jullien Frédéric<sup>2</sup> / Sylvie Baudino<sup>2</sup> / Sana Ajouai<sup>1</sup> / Hassan Ait Mouse<sup>1</sup> / Abdelmajid Ziad<sup>1</sup>

# Phytochemical analysis, cytotoxic and antioxidant activities of *Myrtus communis* essential oil from Morocco

<sup>1</sup> Experimental Oncology and Natural Substances Team, Cellular and Molecular Immuno-pharmacology, Faculty of Science and Technology, Sultan Moulay Slimane University, Beni-Mellal, Morocco, E-mail: ab.zyad2@gmail.com. <https://orcid.org/0000-0002-0371-0927>.

<sup>2</sup> Laboratory of Plant Biotechnology Applied to Aromatic and Medicinal Herbs, Faculty of science and Technology, Jean Monnet University, Saint-Etienne, France

## Abstract:

**Background:** *Myrtus communis* L. is an aromatic evergreen plant common in Morocco. In addition to its culinary uses, it has been used medicinally as a disinfectant, an antiseptic or as a hypoglycemic agent. However, its cytotoxic activity has not been well investigated so far. The current study describes the chemical composition, cytotoxic and antioxidant activities of *Myrtus communis* L essential oil obtained from different regions of Morocco.

**Methods:** *Myrtus communis* essential oils were obtained by hydrodistillation, and analyzed by gas chromatography coupled with mass spectrometry. Cytotoxic activity was evaluated in murine mastocytoma P815 and MCF-7 breast cancer cells, using the MTT assay. In addition, DNA fragmentation was assessed by gel electrophoresis. The antioxidant effect was determined by measuring bleaching of  $\beta$ -carotene with the linoleic acid and the DPPH radical scavenging methods.

**Results:** GC-MS analysis showed high amounts of methyl eugenol (18.7%),  $\alpha$ -terpineol (15.5%) and geranyl acetate (11.64%) in essential oil from the Benslimane region. In contrast, essential oil from Ouazzane was particularly rich in 1,8-cineole (36.3%). The cytotoxicity results showed that MCF-7 cells were more sensitive than P815 cells to the essential oils from Ouazzane and Benslimane regions with  $IC_{50}$  values of 4 and 6.25  $\mu$ g/mL, respectively. Moreover, this cytotoxicity was partly associated with DNA fragmentation, which is one of the characteristics of apoptosis. The tested essential oils did not show strong antioxidant activity.

**Conclusions:** *Myrtus communis* L. essential oil exhibits a weak antioxidant effect, but induced remarkable cytotoxic activity by a mechanism related to apoptosis, suggesting a possible application of the bioactive compounds as natural anticancer compounds.

**Keywords:** antioxidant, apoptosis, cytotoxicity, essential oil, *Myrtus communis*

**DOI:** 10.1515/jcim-2018-0100

**Received:** July 1, 2018; **Accepted:** October 30, 2018

## Introduction

Since ancient history, plants have been important as a source of medicines, flavors and in culinary applications [1, 2]. The World Health Organization states that more than 80% of the world's population, notably in developing countries, use traditional medicine for their primary healthcare. In Morocco, the use of medicinal herbs represents a long history of human interactions with their environment. Traditionally used plant-based medicines contain a wide range of molecules that have been used to treat different diseases, e.g. infections or malaria [3, 4], and they continue to provide numerous opportunities for new drug discoveries.

Processing of these plants for medicinal uses mainly involves extraction of their essential oils (EOs). The latter are considered to be high added value products, and show biological activities ranging from antioxidant capacity to inhibition of tumour proliferation [5, 6]. Indeed, despite the significant advances of modern medicine, there are downsides to conventional drug treatments including high incidence of severe side effects, and development of drug resistance [7].

Abdelmajid Ziad is the corresponding author.  
© 2019 Walter de Gruyter GmbH, Berlin/Boston.

Myrtle (*Myrtus communis* L.) is among the most important medicinal and aromatic species from the Myrtaceae family and it is widely distributed in the Mediterranean regions [8]. In Morocco, the genus *Myrtus* is represented by one species, *M. communis*, which grows wild in Central and Northern Morocco in inland hills, coastal areas and forests areas [9]. Until now, most research focused on its volatile components and its phenolic compounds [10]. The chemical composition of EOs from different regions was described in the literature [11, 12]. Through profiling these oils, different chemotypes can be differentiated based on the amount of myrtenyl acetate and the ratio of  $\alpha$ -pinene to myrtenyl acetate or  $\alpha$ -pinene to cineole [13]. Different parts of the *M. communis* plant contain different compounds such as flavonoids, tannins, and phenolic acid compounds for which antibacterial, antiviral, antifungal, anti-parasitic, and anti-inflammatory activities have been reported [14, 15]. However, few studies have demonstrated the antioxidant activity of myrtle leaf EO [16], while the cytotoxic activity of this EO was not well described. Therefore, the main goal of this work was to investigate the chemical profile, to evaluate the antioxidant properties and the cytotoxic activity of the EO obtained from *M. communis* against cancer cells. The results obtained from this study can help in the development of novel promising therapeutic modalities for cancer.

## Materials and methods

### Material plant

The aerial parts of *M. communis* were harvested in November 2014 from populations located in the Benslimane and Ouzzane regions of Morocco. The plants were identified and stored as a voucher specimens "Herbier FSTBM, LT0020" at the Sultan Moulay Slimane University. After collection, fresh leaves of *M. communis* were shade dried during 2 weeks, at room temperature (25 °C).

### Determination of EO composition

The dried *M. communis* powdered leaves (200 g each sample) were hydrodistilled for 4 h in Clevenger-type apparatus to obtain the EOs. GC/MS analysis of EOs was carried out using an Agilent HP GC (6850) linked to an Agilent HP MS detector (5973) operating in the EI mode. GC conditions were as follows: injector and transfer line temperatures: 250 and 280 °C, respectively; column: DB-5MS fused silica capillary column (30 m  $\times$  0.25 mm, film thickness 0.25 mm); oven temperature: from 60 °C, with an increase of 3 °C min<sup>-1</sup>, to 245 °C (isothermal for 4 min); injection volume: 2  $\mu$ L (hexane solution 0.5%) in split mode; carrier gas: helium at 1.3 mL min<sup>-1</sup>; mass spectra: ionizing recorded at voltage of 70 eV with a scan mass range of 35–250 m/z; Mass unit conditions: ion source temperature: 230 °C. The identification of the EO components was performed using Wiley, National Institute of Standards and Technology NIST 05 and Adams mass spectra databases [17].

### Antioxidant activity

#### Radical scavenging activity assay

The antioxidant activity was carried out in a 96-well microtiter plate using (2,2-diphenyl-1-picrylhydrazyl (DPPH). The essential oils and positive control (vitamin C) were diluted with methanol to prepare sample concentrations equal to 400, 200, 100, 50, 25, 12.5, 6.25, and 3.125  $\mu$ g of dried EO /mL solutions. DPPH 150  $\mu$ L (0.004%) solution was added into each well of the 96-well plates followed by 8  $\mu$ L of the essential oils solution. DPPH 150  $\mu$ L (0.004%) solution was added into each well of the 96-well plates followed by 8  $\mu$ L of the essential oils solution. Then, the plates were incubated at 37 °C for 30 min and absorbance was measured at 540 nm, using ELISA 96-well microplate reader. The assays were performed in triplicate and scavenging activity percentage was calculated by the following formula:

$$\text{DPPH radical scavenging (\%)} = ((A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}) \times 100,$$

where  $A_{\text{blank}}$  is the absorbance of the control reaction (containing all reagents except the test EO), and  $A_{\text{sample}}$  is the absorbance in the presence of the test EO.

### $\beta$ -carotene bleaching assay

EOs antioxidant activity was determined using the  $\beta$ -carotene-linoleic acid test as described by Miller et al. (1971) [18]. The EOs were diluted in ethanol: water (1:1) to a concentration equivalent to 100  $\mu\text{g}/\text{mL}$ . Vitamin C (positive control) or butylated hydroxytoluene (BHT, standard) or water (blank) at the same concentration as the EOs were added to 96-well microplates. Dissolved  $\beta$ -carotene (2.0 mg) in chloroform (1.0 mL) was added to linoleic acid, Tween 80 emulsifier and chloroform. Under a flow of  $\text{N}_2$ , chloroform was evaporated and samples shaken vigorously for 30 min. The resulting emulsion (240  $\mu\text{L}$ ) was added to each well and the zero time optical density was immediately measured at 470 nm using an ELISA 96-well microplate reader. This emulsion solution was incubated for 2 h at 50 °C and the optical density was measured every 15 min.

### Cell culture and cytotoxicity assay

The breast adenocarcinoma (MCF-7) and the murine mastocytoma cell lines were kindly donated by Dr Michel Lepoivre, the laboratory of UMR CNRS 9198, Bât. 430, Paris-Saclay University, France. The cancer cell lines were cultured at 37 °C in a humidified atmosphere containing 5%  $\text{CO}_2$  in modified Eagle's medium (DMEM) complimented with 10% heat inactivated fetal calf serum (Gibco BRL, Cergy Pontoise, France), 0.2% sodium bicarbonate (Sigma) and 1% penicillin G- streptomycin.

Cellular viability was performed using the (3- [4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) (MTT) assay [19]. The starting were harvested at the exponential growth phase ( $4.8 \times 10^4$  cells/mL for MCF7, and  $2 \times 10^5$  cells/mL for P815) were harvested, then washed with phosphate buffered saline (PBS) and seeded in flat-bottomed 96-well microtiter plates. Next, the cells were treated with EOs dissolved in dimethyl sulfoxide (DMSO) at the appropriate concentrations. Control cells were stimulated with DMSO at a final concentration not exceeding 0.5% (v/v). After 48 h of incubation at 37 °C, 5%  $\text{CO}_2$  in a humidified atmosphere, 20  $\mu\text{L}$  of MTT (5 mg/mL in PBS) was added. After 4 h of incubation under these conditions, the generated formazan crystals were dissolved with Hydrochloric acid: Isopropanol (24:1), then the optical density was measured at 540 and 630 nm using Multiskan EX apparatus. The cell inhibition was calculated using the following formula:

$$\% \text{ inhibition} = 100 \times (1 - (\text{OD}/\text{OD}_0)),$$

where  $\text{OD}_0$  and OD are the optical densities of negative control and *M. communis* EOs treated cells, respectively. The cytotoxic activity of *M. communis* EOs against the two cancer cell lines was compared by calculating the  $\text{IC}_{50}$  values (concentration leading to 50% of cell inhibition).

### DNA fragmentation test

P815 cancer cells (approximately  $10^6$  cells/mL) were incubated at 37 °C with 5%  $\text{CO}_2$  for 24 h with the *M. communis* EOs harvested in different regions in Morocco. The concentrations used for this experiment are those generating 50% cell lysis (i.e.  $\text{IC}_{50}$  corresponding concentrations). The treated cells were harvested and lysed by the lysis buffer (10% sodium dodecyl sulfate (SDS), 100 mM Tris HCl, 5 M NaCl, 0.5 M EDTA, and 20 mg/mL Proteinase K). After incubation for 3 h at 37 °C with constant agitation, cell digestion was completed. After precipitation of DNA with isopropanol, the DNA was dissolved at 37 °C with constant agitation in 60  $\mu\text{L}$  of 10 mM tris HCl pH 7.5, 0.1 M EDTA. Finally, 10  $\mu\text{L}$  of the loading buffer (bromophenol blue, ficoll, glycerol, EDTA and xylene cyanol) was added and DNA samples were separated for 4 h (30 mA, 50 V) by horizontal agarose gel electrophoresis on a 1.8% agarose gel stained with ethidium bromide [20]. The gel was visualized on the UV-transilluminator (310 nm). DNA obtained from cells cultured under serum starvation condition was used as a positive control.

### Statistical analysis

Data are expressed as the mean  $\pm$  S.D of three independent experiments. The experiments were performed in triplicates. The significance of differences was assessed using the Student's paired t-test. A p-value less than 0.05 was considered significant. All statistical analyses were carried out using STATISTICA software (TIBCO Statistica™. Version 13.5.0 - 3307 Hillview Avenue. Palo Alto, CA 94304 USA).

## Results

### Chemical profile of the EOs

The EO chemical composition was determined using GC-MS analysis. Twelve compounds were identified, which accounted for 76.95% and 77.88% of the total of *M. communis* EOs from the Benslimane and Ouazzane regions, respectively (Table 1). Both EOs contained a high concentration of monoterpene compounds. The main constituents of *M. communis* EO from the Benslimane region were methyl eugenol (18.7%),  $\alpha$ -terpineol (15.5%), geranyl acetate (11.64%), myrtenyl acetate (8.82%), and 1,8-cineole (8.08%). In the EO of *M. communis* from the Ouazzane region, high amounts of 1,8-cineole (36.37%), methyl-eugenol (9.43%), geranyl acetate (9.3%) and myrtenyl acetate (7.13%) were detected as major constituents (Table 1). See chromatograms of both EOs as Supplemental Material, Figure S1.

**Table 1:** Chemical composition of Benslimane and Ouazzane EO.

Component	RT <sup>a</sup> (mn)	RI <sup>b</sup>	RI <sup>c</sup>	EOs (%)	
				Benslimane	Ouazzane
$\alpha$ -pinene	5.24	930	932	1.35	4.76
myrcene	6.67	987	988	0	0.39
1,8-cineole	8.13	1032	1026	8.08	36.37
$\alpha$ -terpinene	8.96	1056	1054	0	0.21
linalol	10.65	1104	1095	5.82	1.45
terpinen-4-ol	13.82	1181	1174	0.7	0.77
$\alpha$ -terpineol	14.57	1200	1186	15.5	6.96
geraniol	16.75	1252	1249	3.9	0.68
myrtenyl acetate	19.72	1322	1324	8.82	7.13
geranyl acetate	22.11	1378	1379	11.64	9.3
methyl eugenol	23.15	1403	1403	18.7	9.43
caryophyllene oxide	30.11	1577	1582	2.44	0.43
Total				76.95	77.88

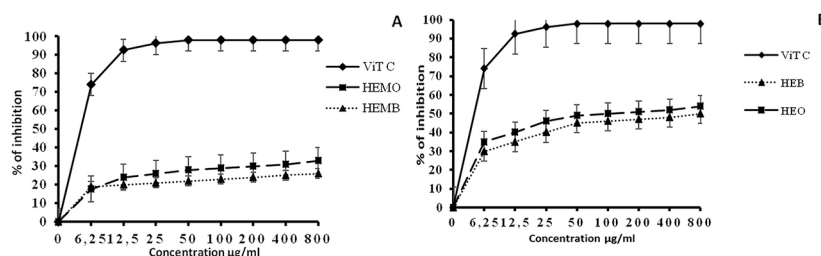
<sup>a</sup>Retention Time.

<sup>b</sup>Retention indices calculated against n-alkanes on DB-5 MS column.

<sup>c</sup>Retention indices referred to in the literature.

### Antioxidant activity

The antioxidant activity of EOs was assessed by both  $\beta$ -carotene-linoleic acid and DPPH assays. The results are presented in Figure 1A and Figure 1B. In the DPPH test system (Figure 1A), the *M. communis* EO from Ouazzane exhibited a similar antioxidant activity compared to EO of *M. communis* from Benslimane. This effect was lower than the Vitamin C used as positive control. In the  $\beta$ -carotene bleaching system (Figure 1B), similar results were obtained to the DPPH assay. In fact, *M. communis* EOs from the Benslimane region displayed a very weak activity with an  $IC_{50}=800 \mu\text{g}/\text{mL}$  of EO from Benslimane region (Figure 1B).



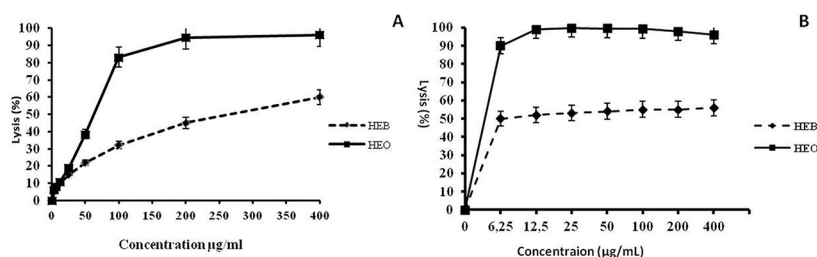
**Figure 1:** Antioxidant activity of *M. communis* EOs.

The antioxidant effect of tested EOs was compared with the synthetic antioxidant Vitamin C (VitC) using the DPPH free radical scavenging assay (A) and the  $\beta$ -carotene-linoleic acid bleaching method (B). Data are the mean  $\pm$  SD of three different experiments performed in triplicates as described in Materials and Methods section.

HEO (essential oil from the Ouazzane region), HEB (essential oil from the Benslimane region).

## Differential cytotoxic activity of *M. communis* EO

The cytotoxic effect of EOs of *M. communis* were tested against P815 and MCF-7 cancer cell lines using the MTT assay. The percentage of viable cells after incubation with different concentrations of EO is shown in Figure 2. Data show that the cytotoxic activity is dose dependent, as the incubation of cancer cells with higher concentrations of EOs for 48 h significantly decreased the viability of these cell lines. The results show that the cytotoxic effects of EO from *M. communis* against MCF-7 cells (Figure 2B) is more pronounced than the cytotoxic effects towards P815 cells (Figure 2A). In addition, the cytotoxic effect of EO from *M. communis* from the Ouazzane region was higher against both cell lines compared to the EOs from *M. communis* from the Benslimane region (Figure 2A, Figure 2B). The  $IC_{50}$  was 4  $\mu\text{g}/\text{mL}$  and 53.9  $\mu\text{g}/\text{mL}$  for EOs from *M. communis* from Ouazzane compared to EO from Benslimane ( $IC_{50}$ =6.25  $\mu\text{g}/\text{mL}$  and 260.0  $\mu\text{g}/\text{mL}$ ) towards MCF7 and P815, respectively.



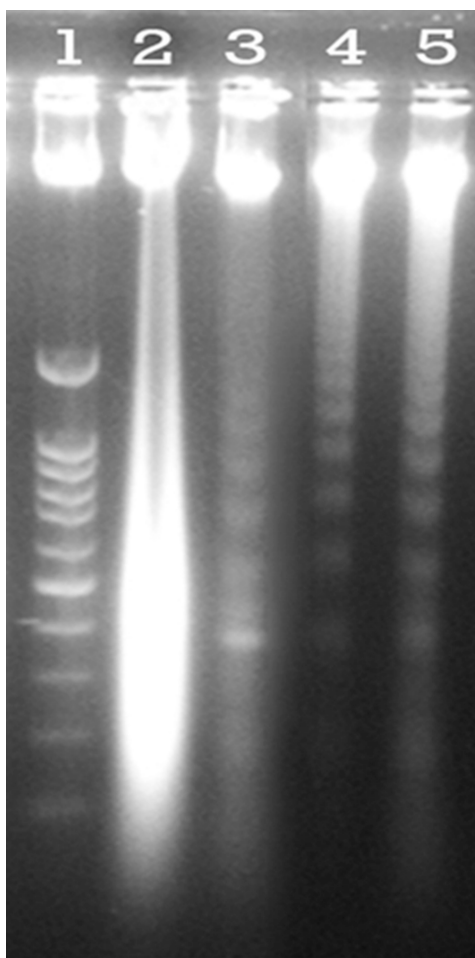
**Figure 2:** Cytotoxic effect of essential oils of Moroccan myrtle against P815 tumor cell P815 (A) and MCF-7 cell lines (B). Cells were stimulated with increasing concentrations of *M. communis* EOs. After 48 h of incubation, cytotoxic activity was calculated as described in the Materials and Methods. Data are represented as mean  $\pm$  SD of three independent experiments.

HEO: essential oil from the Ouazzane region; HEB: essential oil from the Benslimane region).

## Apoptosis induction

DNA fragmentation was analyzed after treatment of P815 cancer cells with EOs from *M. communis* for 24 (Figure 3). The endonucleolytic DNA fragmentation was analyzed by agarose gel electrophoresis and revealed 180–200 bp DNA fragments characteristic for programmed cell death by apoptosis. From this result, we can conclude that *M. communis* EO inhibits P815 cancer cell proliferation through induction of apoptosis.





**Figure 3:** Agarose gel electrophoresis on showing the apoptotic fragmentation of the DNA in the P815 line. Cells treated with  $IC_{50}$  concentrations of EOs (Ouazzane region  $IC_{50} = 53.9 \mu\text{g/mL}$  and Benslimane region  $IC_{50} = 260 \mu\text{g/mL}$ ). 1: Positive Control; 2: Negative Control; 3: Molecular weight marker; 4: EO from the Ouazane region; 5: EO from the Benslimane region.

## Discussion

Chemical analysis showed a predominance of monoterpenes as components in EOs from *M. communis* as shown in Table 1. These results are in agreement with other findings reporting the presence of myrtenyl acetate as one of the main constitutions of *M. communis* EO, in association with  $\alpha$ -pinene and 1,8-cineole [21]. However, different ratios of these compounds were observed in the chemical profiles of EOs derived from different Moroccan regions. This variability in the chemical composition of myrtle extracts could be due to many factors such as the method of extraction and preservation, the soil, the climate and the harvest time [2]. Genetic factors [22] and the vegetative cycle [23] might play also an important role in the EO chemical composition, and thus affect the concentrations of the main constituents.

The antioxidant effect of *M. communis* EOs from both regions, compared to ascorbic acid as a reference was carried out by two complementary assay systems, the  $\beta$ -carotene–linoleic acid bleaching and DPPH free radical scavenging tests (Figure 3A and Figure 3B). We report that the EOs from two Moroccan regions showed little antioxidant activity compared to ascorbic acid.

These findings are not in agreement with those of others who showed a powerful antioxidant effect of tested EOs [24, 25]. This may be due to the high concentration in oxygenated and hydrocarbon monoterpenes present in those EOs [5]. In our experiments, the EOs exhibited weak antioxidant activity as measured by reduction of DPPH free radicals and preventing the linoleic acid oxidation. Such results could be related to the low amounts or the absence of phenolic compounds present in the oil. A weak antioxidant activity was reported by other authors and they concluded that it was due to the low phenol and flavonoid content of the plant [26]. Furthermore, the presence of methyl eugenol in the EOs from our samples might be also responsible for the weak

antioxidant effect observed in this work. As reported by Joshi et al 2013, methyl eugenol has been shown to possess a much weaker antioxidant activity, due to delocalized electron(s) or proton(s) of methyl eugenol [27]. In the same study, the authors also reported that the presence of the eugenol, epi-cubebol, epi- $\alpha$ -cadinol, cubebol cineol-1,8, 10-epi-cubebol, 1,10-di-epi-cubebol and,  $\alpha$ -terpineol in the EOs oil composition could instigate the weak antioxidant activity [27].

The studied EOs demonstrated a significant dose dependent cytotoxic effect against the tumor cell lines. Our results are in agreement with other reports demonstrating that at 100  $\mu\text{g}/\text{mL}$  concentration, *M. communis* EO exhibited an 81.4% inhibition of breast cancer cells, while at a concentration of 200  $\mu\text{g}/\text{mL}$  the inhibition was 95.2% and 67% for breast and prostate tumor cell lines, respectively [28]. Our study corroborates these observations and adds that the same inhibition of cell viability is not obtained when the P815 cell line is used as a target. Interestingly, we reported here that MCF-7 cells were more sensitive than P815 cells to the essential oils from the Ouazzane and Benslimane regions. Taken together, our results underline a selective cytotoxic effect of *M. communis* EO against cancer cells. Thus, we can suggest that the differential cytotoxic activity of these EOs is associated not only with their chemical composition profile, but also to the type of cancer cell lines. In order to understand the molecular pathways related with the observed cytotoxic effect of *M. communis*, a DNA fragmentation assay was performed using the P815 cancer cells. Our result demonstrated that this cytotoxicity is partly associated with DNA fragmentation induction, characteristic of apoptotic death (Figure 3).

There are numerous research studies regarding the occurrence of apoptosis in the extracts of plants from the Myrtaceae family, including the plant *M. communis*, and their different compounds [27].

Our finding is in accordance with previous studies showing apoptosis induction in different tumor cell lines by *M. communis* EO [15, 29]. Myrtucommulone (derived from *M. communis* leaves) was shown to increase the activity of caspases 3 and 9, and to release nucleosomes into the cytosol and cytochrome c from mitochondria, causing cleavage of Poly ADP-Ribose Polymerase (PARP) and DNA fragmentation related to cell death by apoptosis [29].

## Conclusions

In conclusion, *M. communis* EO exhibited weak antioxidant effect and showed significant cytotoxic activity in different cell types by a mechanism related to apoptosis. Hence *M. communis* EO may be considered as a source for potential anticancer agents. It would be interesting to investigate the role that different molecules of the EO play in the effect showed here, to determine which of them could have potential anti-cancer activities, either alone or in combination.

**Author contributions:** All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

**Research funding:** This work received financial support from Lalla Salma Foundation: Prevention and Treatment of Cancer, Rabat, Morocco (Project 9/2013).

**Employment or leadership:** None declared.

**Honorarium:** None declared.

**Competing interests:** The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

## References

- [1] Hornsey IS. Alcohol and its role in the evolution of human society. Cambridge, UK: Royal Society of Chemistry, 2012
- [2] Tilaoui M, Ait Mouse H, Jaafari A, Ziad A. Comparative phytochemical analysis of essential oils from different biological parts of artemisia herba alba and their cytotoxic effect on cancer cells. PloS One. 2015;10:e0131799.
- [3] Tilaoui M, Ait Mouse H, Jaafari A, Ziad A. Differential effect of artemisinin against cancer cell lines. Nat Prod Bioprospecting. 2014;4:189–96.
- [4] Ziad A, Tilaoui M, Jaafari A, Oukerrou MA, Ait Mouse H. More insights into the pharmacological effects of artemisinin. Phyto Res. 2017 11 28;32:216–29. DOI: 10.1002/ptr.5958
- [5] Lai P, Rao H, Gao Y. Chemical composition, cytotoxic, antimicrobial and antioxidant activities of essential oil from anthriscus caucalis M. Bieb Crown in China. Rec Nat Prod. 2018;12:10



- [6] Tilaoui M, Ait Mouse H, Jaafari A, Aboufatima R, Chait A, Ziad A. Chemical composition and antiproliferative activity of essential oil from aerial parts of a medicinal herb *Artemisia herba-alba*. *Rev Bras Farmacogn*. 2011;21:781–85.
- [7] Chu E, DeVita JVT. *Physicians' cancer chemotherapy drug manual* 2018. USA: Jones & Bartlett Learning 2017
- [8] Zilkah S, Goldschmidt EE. Myrtle (*Myrtus communis* L.)—A native Mediterranean and cultured crop species. *Medicinal and Aromatic Plants of the Middle-East*. Zohara Yaniv, Nativ Dudai(eds.). Heidelberg Springer, 2014:253–67.
- [9] Bouzabata A, Casanova J, Bighelli A, Cavaleiro C, Salgueiro L, Tomi F. The Genus *myrtus* L. in Algeria: composition and biological aspects of essential oils from *M. communis* and *M. nivellei*: a review. *Chem Biodivers*. 2016;13:672–80.
- [10] Wannas WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, et al. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. *Food Chem Toxicol*. 2010;48:1362–70.
- [11] Lawrence BM *Progress in essential oils* 1993.
- [12] Ben Hsouna A, Hamdi N, Miladi R, Abdelkafi S. *Myrtus communis* essential oil: chemical composition and antimicrobial activities against food spoilage pathogens. *Chem Biodivers*. 2014;11:571–80.
- [13] Boelens MH, Jimenez R. The chemical composition of Spanish myrtle oils. Part II. *J Essent Oil Res*. 1992;4:349–53.
- [14] Alipour G, Dashti S, Hosseinzadeh H. Review of pharmacological effects of *Myrtus communis* L. and its active constituents. *Phytother Res*. 2014;28:1125–36.
- [15] Shahnazi M, Azadmehr A, Jondabeh MD, Hajiaghaee R, Norian R, Aghaei H, et al. Evaluating the effect of *Myrtus communis* on programmed cell death in hydatid cyst protoscolices. *Asian Pac J Trop Med*. 2017;10:1072–76.
- [16] Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I. Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. *Phytochemistry*. 2006;67:1249–55.
- [17] P R AD. *Identification of essential oil components by gas chromatography/mass spectrometry*, 4th ed. Carol Stream, Ill: Allured Pub Corp, 2007.
- [18] Miller HE. A simplified method for the evaluation of antioxidants. *J Am Oil Chem Soc*. 1971;48:91–91.
- [19] Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65:55–63.
- [20] Laird PW, Zijderveld A, Linders K, Rudnicki MA, Jaenisch R, Berns A. Simplified mammalian DNA isolation procedure. *Nucleic Acids Res*. 1991;19:4293.
- [21] Mimica-Dukić N, Bugarin D, Grbović S, Mitić-Ćulafić D, Vuković-Gačić B, Orčić D, et al. Essential oil of *Myrtus communis* L. as a potential antioxidant and antimutagenic agents. *Molecules*. 2010;15:2759–70.
- [22] Echeverrigaray S, Agostini G, Atti-Serfina L, Paroul N, Pauletti GF, Atti Dos Santos AC. Correlation between the chemical and genetic relationships among commercial thyme cultivars. *J Agric Food Chem*. 2001;49:4220–23.
- [23] Hancı SS, Sahin S, Yılmaz L. Isolation of volatile oil from thyme (*Thymbra spicata*) by steam distillation. *Mol Nutr Food Res*. 2003;47:252–55.
- [24] Cherrat L, Espina L, Bakkali M, García-Gonzalo D, Pagán R, Laglaoui A. Chemical composition and antioxidant properties of *Laurus nobilis* L. and *Myrtus communis* L. essential oils from Morocco and evaluation of their antimicrobial activity acting alone or in combined processes for food preservation. *J Sci Food Agric*. 2014;94:1197–204.
- [25] Noori S, Zeynali F, Almasi H. Antimicrobial and antioxidant efficiency of nanoemulsion-based edible coating containing ginger (*Zingiber officinale*) essential oil and its effect on safety and quality attributes of chicken breast fillets. *Food Control*. 2018;84:312–20.
- [26] Jia J, Ma H, Zhao W, Wang Z, Tian W, Luo L, et al. The use of ultrasound for enzymatic preparation of ACE-inhibitory peptides from wheat germ protein. *Food Chem*. 2010;119:336–42.
- [27] Joshi RK. Chemical composition, in vitro antimicrobial and antioxidant activities of the essential oils of *ocimum gratissimum*, *O. Sanctum* and their major constituents. *Indian J Pharm Sci*. 2013;75:457–62.
- [28] Stevens N. *Essential oil cancer research*. Utah: Spanish Fork. 2010:1–16.
- [29] Tretiakova I, Blaesus D, Maxia L, Wesselborg S, Schulze-Osthoff K, Cinatl J, et al. Myrtucommulone from *Myrtus communis* induces apoptosis in cancer cells via the mitochondrial pathway involving caspase-9. *Apoptosis*. 2008;13:119–31.

**Supplementary Material:** The online version of this article offers supplementary material (DOI:<https://doi.org/10.1515/jcim-2018-0100>).