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Cytotoxic activity of Moroccan *Melissa officinalis* leaf extracts and HPLC-ESI-MS analysis of its phytoconstituents

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Abstract

Background: *Melissa officinalis* L. is a medicinal and aromatic plant traditionally used in Morocco to treat a weave range of illness. The aim of our study was to evaluate cytotoxic activity of Moroccan *Melissa officinalis* leaf extracts against three human cancer cell lines, namely, MCF7, LNCAP and PC3 and to reevaluate its phytochemicals.

Results: The dichloromethane extract was found to be the most active cytotoxic extract, decreasing cell viability in a dose-dependent manner, especially against the breast MCF7 cell line. The IC_{50} values for the dichloromethane extract against MCF7, LNCAP, and PC3 cell lines were 30.90, 71.21, and 173.93 μg/mL respectively whereas the corresponding IC_{50} values for the ethanol extract were 35.52, 136.40, and 237.82 μg/mL. An update of the chemical profiles of these organic extracts was conducted by GC-MS, HPLC, and HPLC-ESI-MS, and the quantity of total polyphenolic compounds (on a dry weight basis) was 61.84 g/kg and 2.86 g/kg in the ethanol and dichloromethane extracts, respectively. The major polyphenolic compounds identified in the ethanol extract were 3,4-dihydroxyphenyl lactic acid (I), 3,4-dihydroxybenzoic acid (II), caffeic acid (III), luteolin-7-O-glucoside (IV), rosmarinic acid glucoside (V), methyl caffeate (VI), rosmarinic acid (VII), isolithospermic acid (VIII), methyl rosmarinate (IX), lithospermic acid (X), methyl isolithospermic acid (XI), and methyl lithospermic acid (XII). Of these, 3,4-dihydroxyphenyl lactic acid (I), isolithospermic acid along with its methyl ester derivative are reported in *Melissa officinalis* leaves extract for the first time. In addition, *o*-tyrosol (XIII), methyl hydroxyphenyl acetic acid (XIV), and *cis*-rosmarinic acid (XV) were also detected in the DCM extracts. In the *n*-hexane extracts LCFA (palmitic, linolenic, linoleic, and stearic acids), sterols (campesterol, β-sitosterol, and stigmasterol), and the vitamins (α- and β-tocopherol) were detected and identified.

Conclusion: These results indicated that *Melissa officinalis* L extracts possess a potent cytotoxic effect against human cancer cell lines and the richness of this herb in bioactive molecules justifying its use in traditional Moroccan pharmacopeia.

Keywords: *Melissa officinalis* L., Cytotoxicity, GC-MS, HPLC, HPLC-ESI-MS, Lithospermic acid, Rosmarinic acid

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Background

The Moroccan population is renowned for its traditional medicine and know-how based on medicinal plants. Its geographical position and its varied bioclimates allow Morocco to enjoy a rich and diversified flora with about 7000 identified species [1]. Of these, *Melissa officinalis*, known locally as "Hbak tranj" extracts of which are traditionally used in Moroccan folk medicine, to calm nerves, and increase body strength [2]. A herbal infusion of the leaves is renowned for its digestive and antispasmodic properties.

Melissa officinalis L. (Lamiaceae) is a perennial herb with a square stem, erect branched, growing as a tuft and is generally 30 to 80 cm in height [3]. Native to the Mediterranean basin, *Melissa officinalis*, also named lemon balm, honey balm, garden balm, and bee balm, is characterized by the lemon scent of the crumpled leaves. It is since ancient times renowned for its healing properties and extensive use in aromatherapy as well as in the food industry [4]. Several therapeutic benefits and pharmacologic investigations unveiled several biologic activities such as antioxidant [5], antidepressant [6], antimicrobial [7], and anti-inflammatory properties [8]. Furthermore, anticancer capacities have been attributed to the use of *Melissa officinalis* [9] and some interesting in vitro studies have shown that *Melissa officinalis* extracts inhibit the growth of colon cancer cells [10, 11].

From a phytochemical point of view, *Melissa officinalis* has been well studied and a phytochemical investigation has revealed the presence of phenolic acids, tannins, flavonoids, triterpenoids, along with volatile compounds [12]. Examples of phytochemicals include citral, a major component of *Melissa officinalis* essential oil which revealed anticancerous activity on large human tumor cell lines [13]. Components of the essential oil are known for their spasmolytic and antimicrobial effects [14]. Furthermore, and among other lamiaceae species, *Melissa officinalis* is a natural source of rosmarinic acid, and a handful of studies have reported its health beneficial effects [15]. Rosmarinic acid has been in addition described as a cytotoxic agent against different human tumor cell lines [16], as well as displaying antiviral and potent antioxidant properties [17]. Currently, special attention is given to the components of *Melissa officinalis* in the food industry because of their significant antimicrobial and antioxidant properties [18].

In this study, we focused on the leaves of *Melissa officinalis*, which are the botanical parts included in pharmacopeia, we then evaluated the cytotoxic effect of leaf extracts growing wild in Morocco against human cancer cell lines, and we next investigated the phytochemical composition of the extracts. To the best of our knowledge, the cytotoxic capacity of Moroccan *Melissa officinalis* leaf extracts has not been conducted until now.

Methods

Plant material

Based on ethnopharmacological data and using the help of traditional medical practitioners, fresh leaves of *Melissa officinalis* L. were collected, in January 2018, in Dhamna Village, located 10 km from the city of El Jadida, Middle Morocco. The plant was identified with botanist of the Laboratory of Quality control in Bioindustry and Bio-active Molecules, Faculty of Science, Chouaib Doukkali University, a voucher specimen (no. RAB76712) was deposited in the Herbarium of Botany Department of Scientific Institute of Rabat.

Leaves were dried at room temperature and ground to a fine homogeneous powder using an electric grinder prior to extraction.

Preparation of plant extracts

Soxhlet extractions of *Melissa officinalis* leaves (40 g) were conducted with *n*-hexane and dichloromethane (600 mL) for 8 h. The ethanol extract was obtained by maceration of 10 g of powdered material in 100 mL of ethanol for 7 days. The extracts were concentrated to dryness by rotary evaporation and the residues were stored at 4 °C for subsequent experiments.

Chemicals and reagents

Acetic acid, acetonitrile, *n*-hexane, lithospermic acid, and methanol were obtained from E. Merck (Darmstadt, Germany). Caffeic acid, 3,4-dihydroxyphenyl lactic acid, 3,4-dihydroxybenzoic acid, luteolin-7-O-glucoside, methyl caffeate, and rosmarinic acid from Extrasynthese (Lyon Nord, Genay, France). All solutions were made up in either doubly distilled water, or methanol, unless otherwise stated.

Gas chromatography-mass spectrometry (GC-MS)

This was conducted by the methods of Owen et al. (2000) on an Agilent 5973 mass quadruple spectrometer coupled to an Agilent 6890 gas chromatograph [19].

Analytical HPLC

Analytical HPLC was conducted as described by us previously [20].

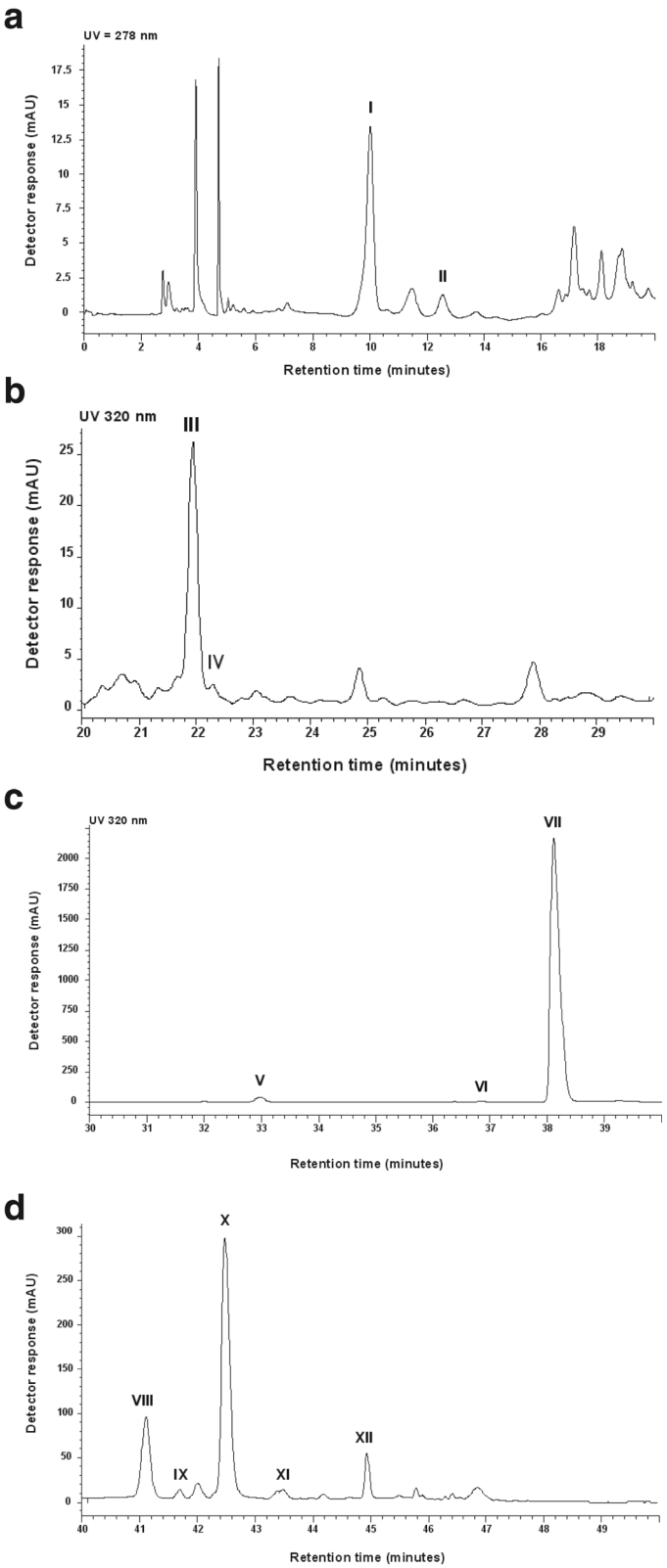

Fig.1 (See legend on next page.)

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Fig.1 a Analytical HPLC chromatogram of an ethanol extract of *Melissa officinalis* (0–20 min). I, 3.4-dihydroxyphenyl lactic acid; II, 3.4-dihydroxybenzoic acid. b Analytical HPLC chromatogram of an ethanol extract of *Melissa officinalis* (20–30 min). III, caffeic acid. c Analytical HPLC chromatogram of an ethanol extract of *Melissa officinalis* (30–40 min). V, rosmarinic acid glucoside. VI, methyl caffeate. VII, rosmarinic acid. d Analytical HPLC chromatogram of an ethanol extract of *Melissa officinalis* (40–50 min): VIII, isolithospermic acid; IX, methyl rosmarinate; X, lithospermic acid; XI, methyl isolithospermic acid; XII, methyl lithospermic acid

HPLC-ESI-MS

HPLC-ESI-MS was conducted exactly as assayed by us previously [21] on an Agilent 1100 HPLC coupled to an Agilent single-quadrupole mass-selective detector (HP 1101; Agilent Technologies, Waldbronn, Germany). Chromatographic separations of extracts were dissolved in methanol and HPLC-MS separations were conducted using a column of the same type and dimensions as for analytical HPLC (Phenomenex, Aschaffenburg, Germany).

Phenolic compounds were detected by their UV absorbance (A) at 278 and 340 nm at 30 °C. Negative-ion mass spectra, were generated under the following conditions: fragmentor voltage, 100; capillary voltage, 2500 V; nebulizer pressure, 30 psi; drying gas temperature, 350 °C; m/z scan range, 100–1500 D. Positive-ion spectra, were generated under the following conditions: fragmentor voltage, 200; capillary voltage, 1500 V; nebulizer pressure, 30 psi; drying gas temperature, 350 °C; m/z scan range, 100–1500 D. For HPLC-ESI-MS-MS experiments, in negative-ion mode, the fragmentor voltage was increased to 300. Quantitation of the polyphenolic compounds was conducted against standard curves (optical absorbance vs. concentration) in the range 0.05–1.0 mM (50, 100, 250, 500, 750, and 1000 μM) prepared using the following authentic commercial samples, namely caffeic acid, 3,4-dihydroxyphenyl lactic acid, 3,4-dihydroxybenzoic acid, lithospermic acid, luteolin-7-O-glucoside, methyl caffeate and rosmarinic acid. *Cis*-rosmarinic acid, methyl rosmarinate, and rosmarinic acid glucoside were quantitated against the standard curve of rosmarinic acid, whereas isolithospermic acid, methyl isolithospermate, and methyl lithospermate were quantitated against the standard curve of lithospemic acid with relevant molecular weight corrections. Instrument control and data handling were performed with the same software as for analytical HPLC.

Cell culture

Human prostate adenocarcinoma PC3 and LNCAP and breast adenocarcinoma MCF7 cancer cell lines were used in this study. Cells were kindly provided by Dr. L'Houcine Ouafik (Laboratoire de transfert d'oncologie, Marseille). PC3, and MCF7 cells were maintained in DMEM medium and LNCAP cells were cultured in RPMI medium. The medium was supplemented with 10% (v/v) fetal calf serum and 1% penicillin/streptomycin mixture (10,000 IU/mL). All cell lines were kept

Table 1 Polyphenolic compounds identified in *Melissa officinalis* ethanol leaf extracts by HPLC-ESI-MS in negative ion mode

Peak no.	R_t (min)	Phenolic compound	UV-Vis maxima (nm)	$[M-H]^-$	$[2M-H]^-$
I	10.04	3,4-dihydroxyphenyl lactic acid	230, 284	197.1	395.2
II	12.56	3,4-dihydroxybenzoic acid	225, 260, 295	153.1	307.1
III	21.94	caffeic acid	220, 240, 295(sh), 325	179.2	359.0
IV	22.03	luteolin-7-O-glucoside	250, 345	447.3	895.4
V	32.98	rosmarinic acid glucoside	220, 280, 325	521.1	1043.2
VI	36.85	methyl caffeate	225, 290 (sh), 320	193.1	387.0
VII	38.11	rosmarinic acid	220, 280, 325	359.1	719.2
VIII	41.11	isolithospermic acid	220, 285 (sh), 320	537.1	1075.2
IX	41.69	methyl rosmarinate	220, 280, 325	373.1	747.2
X	42.48	lithospermic acid	225, 285 (sh), 325	537.1	1075.2
XI	43.49	methyl isolithospermate	225, 285 (sh), 325	551.1	1103.2
XII	44.94	methyl lithospermate	225, 285 (sh), 325	551.1	1103.2

R_t retention time

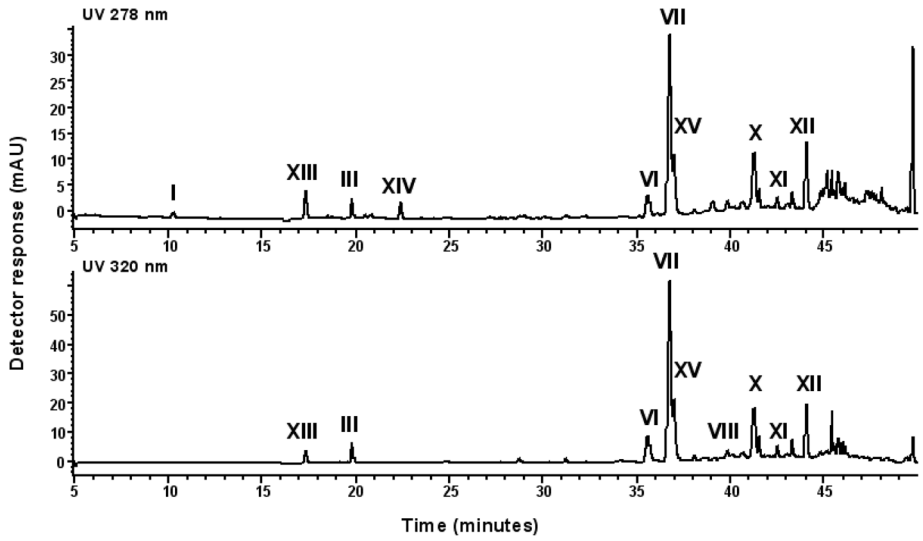

Fig. 2 Analytical HPLC chromatogram of an DCM extract of *Melissa officinalis* at 278 nm

under standard conditions of temperature (37 °C), humidity (95%) and carbon dioxide (5%), and subcultured at 80% confluency.

Evaluation of cell viability by MTT assay

The cytotoxic effect of ethanol and dichloromethane extracts from *Melissa officinalis* on three tumor cell lines was determined by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) colorimetric assay [22]. Briefly, cells in the exponential growth phase were plated in 96-multiwell plates, at a cellular density of 8000 cells/well in 0.1 mL medium. After 24 h, 100 μL of fresh medium, containing serial concentrations ranging from 12.5 to 400 μg/mL of each extract (dissolved in DMSO) was added to the cells and incubated for 72 h at 37 °C. At the end of treatment, 10 μL MTT (5 mg/mL) were added to each well, and plates were reincubated for an additional 4 h at 37 °C. The purple-blue MTT formazan precipitate was dissolved in 100 μL DMSO. The reduced MTT was spectrophotometrically analyzed at 570 nm using a microplate reader. Untreated cells were considered as a negative control, mitomycin C was used as a positive control. Experiments were conducted in duplicate. The percentage of cytotoxicity and cell viability were calculated using following equations:

$$\%\text{Viability} = 100 - \%\text{Cytotoxicity}$$

$$\%\text{Cytotoxicity} = 1 - (\text{mean absorbance of treated cells} / \text{mean absorbance of negative control}).$$

Statistical analysis

The data represented in cytotoxic study are mean SEM of two identical experiments made in duplicates; the statistical differences between the treatments and the positive control were tested by One-way analysis of variance (ANOVA), followed by a multiple comparison to assess the difference of the IC50 values of the same extract on different cell lines. $p < 0.05$ was considered to be statistically significant. The IC_{50} calculations, statistical analysis and graphs plots were done using Graph Pad Prism Data Editor for Windows, Version 6.0 (Graph Pad software Inc., San Diego, CA).

Table 2 Additional compounds identified in *Melissa officinalis* dichloromethane leaf extracts by HPLC-ESI-MS in negative ion mode

Peak no.	R_t (min)	Phenolic compound	UV-Vis maxima (nm)	$[M-H]^-$	$[2M-H]^-$
XIII	17.31	*o*-tyrosol	280, 310	137.0	275.2
XIV	22.41	methyl hydroxyphenyl acetic acid	278	181.2	363.0
XV	36.99	*cis*-rosmarinic acid	220, 280, 325	359.1	719.2

R_t retention time

Results

Phytochemical profile of *Melissa officinalis* extracts

The percentage of extracts by soxhlet apparatus are respectively 37, 4.22, and 2.35 % for ethanol, dichloromethane, and hexane extracts.

The compartmentalized HPLC chromatograms (at various wavelengths) corresponding to the ethanol extract are shown in Fig. 1a–d. In addition, the MS and UV information for the separated components are summarized in Table 1.

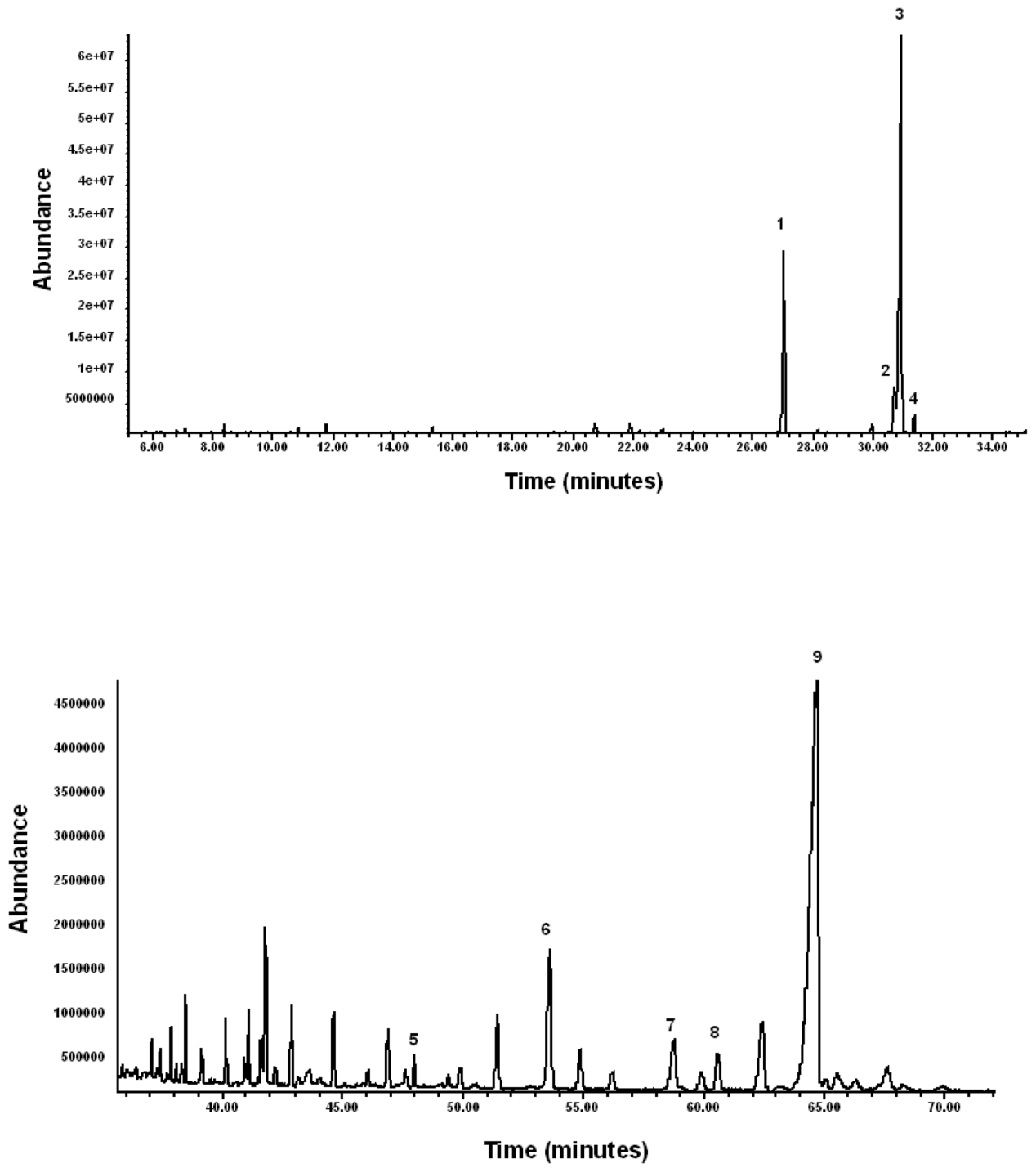

Fig. 3 Chromatogram (GC-EI-MS) of the hexane extract of *Melissa officinalis* following derivatization with BSTFA

The HPLC chromatogram at two wavelengths respectively equal to 280 and 320 nm of the DCM extract are depicted in Fig. 2. The UV and mass spectral characteristics of three metabolites which were only detected in the dichloromethane extracts, identified as *o*-tyrosol, methyl hydroxyphenyl acetic acid, and *cis*-rosmarinic acid are summarized in Table 2.

The chromatogram of the *Melissa officinalis n*-hexane extract obtained by GC-EI-MS after derivatization with BSTFA is depicted in Fig. 3. The main metabolites detected were long chain fatty acids such as palmitic and linoleic acid, sterols such as stigmasterol, campesterol, and □-sitosterol; and the vitamers □- and □-tocopherol. The GC-EI-MS data for the TMS-derivatives is summarized in Table 3. The structures of the polyphenolic compounds and hexane extract sterols and vitamers are depicted in the Figs. 4 and 5, respectively.

Quantitation of polyphenolic compounds in the ethanol and dichloromethane extracts are given in Tables 4 and 5, respectively.

Cytotoxicity studies

The cytotoxic effect of medicinal plant extracts generally leads to changes in the morphology of treated cells that can be visualized under phase-contrast inverted microscope [23]. Treatment of three selected human cell lines with increasing concentrations of *Melissa officinalis* dichloromethane and ethanolic extracts, clearly causes cellular morphological changes, such as detachment and loss of anchorage properties, compared to untreated cells, which retain their anchoring properties, attesting that treated cells went through cell death with pronounced effect on breast MCF7 cells, compared to LNCaP and PC3 cells (data not shown). We next evaluated the cytotoxic effect of different extracts against the three human cancerous lines by MTT assay. In this assay, cell viability was assessed by the ability of viable cells to reduce MTT to formazan. All cell lines were treated for 72 h with the three extracts in concentrations ranging from 400 to 12.5 μg/mL and IC_{50} for each extract was determined.

Our results showed that dichloromethane extract (Fig. 6a) and ethanolic extract (Fig. 6b) extracts decreased cell viability and induced cell growth inhibition in a dose-dependent manner, However, cytotoxic effect of dichloromethane extract was more remarkable than ethanolic extract, with values of IC_{50} ranged from 30.90 ± 1.462 μg/mL and 35.52 ± 0.649 μg/mL in breast MCF7 cells, and 71.21 ± 1.105 and 136.4 ± 0.814 in prostate LNCaP cells, respectively. In PC3 cells, the impact of Dichloromethane extract and ethanol extract was less pronounced with IC_{50} ranging from 173.93 ± 1.07 to 237.82 ± 2.353 μg/mL, respectively. Figure 6c represents the cells line viability for 72 h with hexanic extract which have a remarkable effect on MCF7 cells, a moderate one in LNCaP and no effect against PC3 cells. The IC_{50} values recorded in different cell lines are reported in Table 6.

Table 3 Chemical composition of the *Melissa officinalis* hexane extract

Compound	Retention time (min.)	Mass of silylated compounds	Molecular peaks
Palmitic acid	27.04	328	256
Linoleic acid	30.65	352	280
Linolenic acid	30.91	350	278
Stearic acid	31.37	356	284
β-tocopherol	49.89	488	416
α-tocopherol	53.63	502	430
Campesterol	58.71	472	400
Stigmasterol	59.97	484	412
β-Sitosterol	64.69	486	414

Discussion

Cancer is the second leading cause of death, and is responsible for an estimated 9.6 million deaths in 2018 and globally, about 1 in 6 deaths is due to cancer. In recent decades, the search for new anti-cancer agents has focused on medicinal plants and their derived compounds [24]. Morocco has a rich and diverse flora that deserves a scientific valorization. In this context, we have targeted the study of the cytotoxic effect of *Melissa officinalis* organic extracts which have been widely used for the treatment of several types of cancers [9]. *Melissa officinalis* has also been shown to be rich in antioxidants which may play a vital role in the prevention and treatment of cancer [25]. Breast and prostate cancer are among the most prevalent cancers known, *prostate cancer* is the second *most common* cancer in men, whereas *breast cancer* is the *most common cancer* in women *worldwide* [26].

Our results are in accordance with data reported for *Melissa officinalis* polyphenolic content from a study conducted in Portugal [17] but add some further important metabolites such as isolithospermic acid and its methyl ester form along with 3,4-dihydroxyphenyl lactic acid.

From a phytochemical study, our data add usuful informations about the chemical profile, *Melissa*

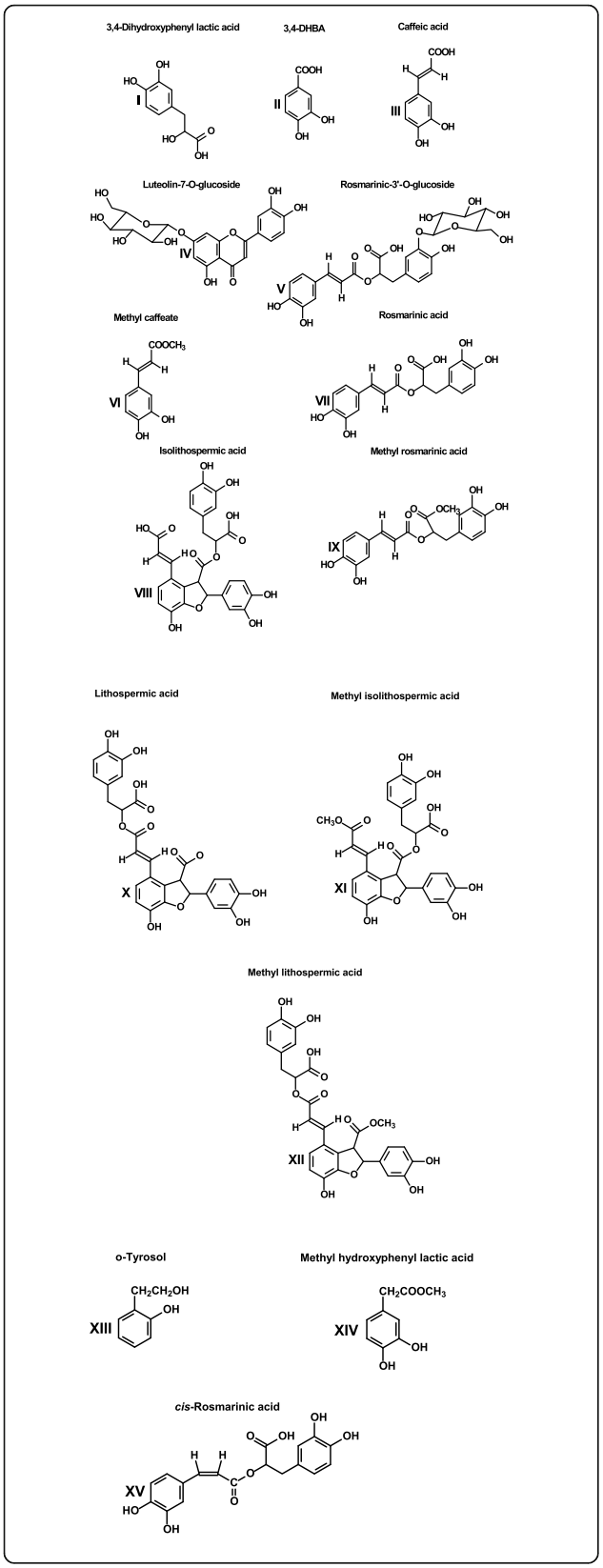

Fig. 4 Structures of the polyphenolic compounds detected in the dichloromethane and ethanol extracts of *Melissa officinalis*. **I** 3,4-dihydroxyphenyl lactic acid, **II** 3,4-dihydroxybenzoic acid, **III** caffeic acid, **IV** luteolin-7-O-glucoside, **V** rosmarinic acid glucoside, **VI** methyl caffeate, **VII** rosmarinic acid, **VIII** isolithospermic acid, **IX** methyl rosmarinate, **X** lithospermic acid, **XI** methyl isolithospermate, **XII** methyl lithospermate, **XIII** o-tyrosol, **XIV** methyl hydroxyphenyl acetic acid, and **XV** cis-rosmarinic acid

officinalis ethanol extract is a rich source of polyphenolic compounds, more particularly, it is a rich source of rosmarinic acid but also lithospermic acid which is caffeic acid trimer, and its methyl forms and isomers, the lipophilic compounds of *Melissa officinalis*, include essential fatty acids, sterols (sitosterol, stigmasterol and campesterol), and tocopherols such as the form alpha and beta.

We have expected a potential cytotoxic effect of *Melissa officinalis* extracts, on breast and prostate human tumor cell lines, we then selected prostate PC3, LNCaP, and breast cancer MCF7 cell lines which are known for their metastatic potential, prostate PC-3 cells have high metastatic potential whereas prostate LNCAP and breast MCF7 cells have lower metastatic potential [27]. In previous report, Jahanban-Esfahlan et al. (2015) evaluated in vitro the antiproliferative effect of extracts of *Melissa officinalis* on a variety of human cancerous cell lines, highlighting the sensitivity of the MCF7 breast line compared to the other tested cell lines. The authors also observed that the hydroalcoholic extract of *Melissa officinalis* inhibited in a dose-independent manner the proliferation of ovarian SKOV3 cells, suggesting optimal doses and tolerated high doses. Moreover, the antiproliferative effect of *Melissa officinalis* seems to be tumor type specific [28]. In another report, Saraydin et al. (2012) showed that methanol *Melissa officinalis* extracts inhibit cell proliferation of breast MCF-7, MDA-MB-468 and MDA-MB-231 cells, with an IC_{50} values ranging between 17 and 19 μg/mL. In this study, authors have performed *in vivo* analysis by immunohistochemistry for caspase 7 protein in the tumoral tissue sections of induced mammary tumors in rats and Tunnel assays for detecting apoptotic cells. Compared with untreated control group, treated rats recorded expression of caspase-7 protein and TUNEL-positive cells as well as a mean tumor volume inhibition ratio was about 40%, suggesting the potent antitumoral effect of *Melissa officinalis* against breast cancer [29]. Other molecular signaling pathways have been also described including induction of apoptosis through formation of ROS in colon carcinoma cell [10]. Against breast

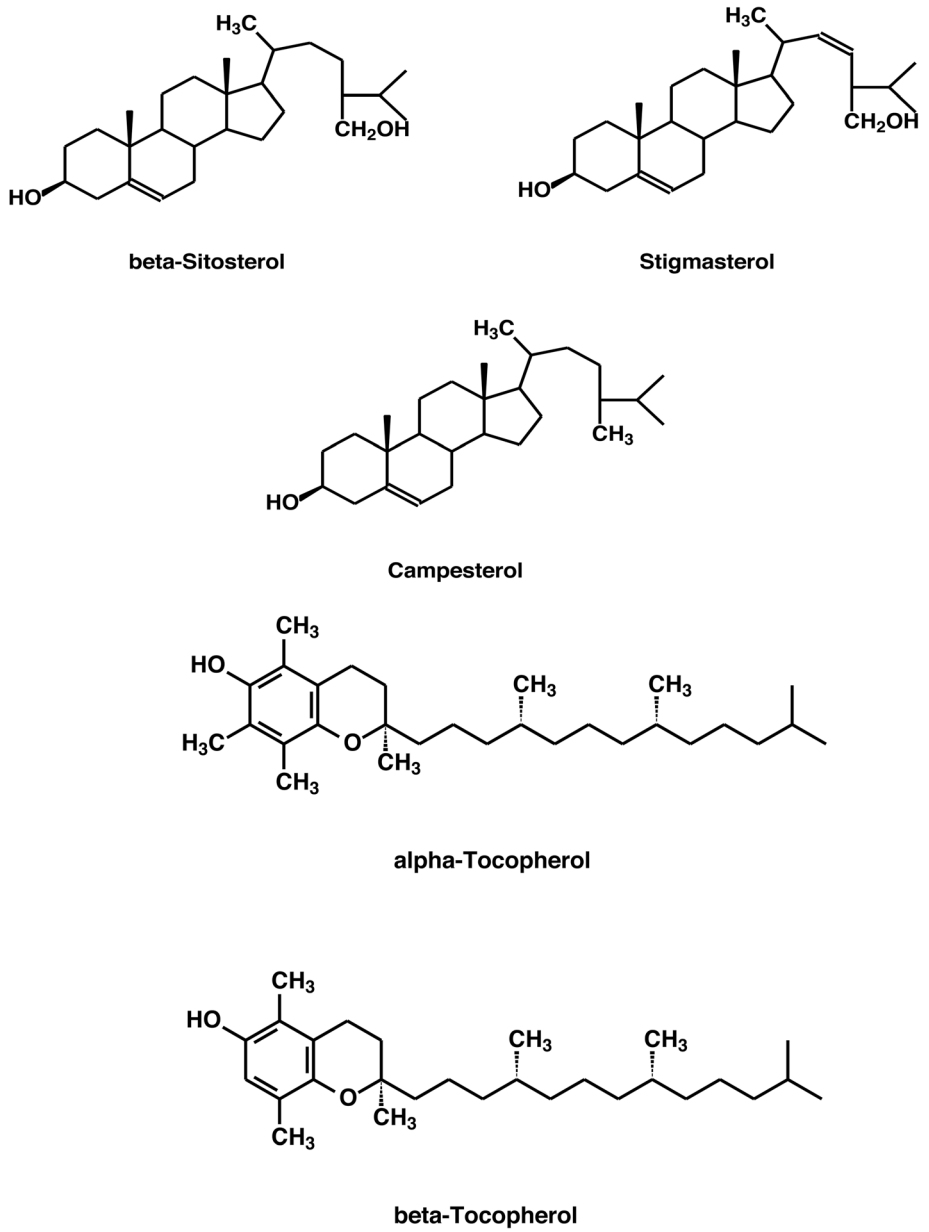

Fig. 5 Structures of the major vitamers and sterols detected in hexane extract of *Melissa officinalis* leaves

cancer cells MDA-MB-231, *Melissa officinalis* ethanolic extract exerted a cytotoxic effect on breast cancer cells (MDA-MB-231) even at low concentrations, with an IC_{50} value of 301.4 ± 10.26 μg/mL [11, 30]. The dichloromethane extract significantly induced apoptosis in leukemia cell line, K562 via upregulation of Fas and Bax mRNA expression and increasing the Bax/Bcl-2 ratio, indicating its capacity in activating both extrinsic and intrinsic pathways of apoptosis. *n*-hexane extracts inhibits considerably K562 and Jurkat cell proliferation with no change in expression of genes involved in apoptosis, this indicate that induction of apoptosis was not responsible for cell growth inhibition [31]. Apoptosis was probably induced by the lipophilic compounds present in dichloromethane and *n*-hexane extracts. In the elegant report of Moacă et al. (2018), authors have demonstrated that *Melissa officinalis* extracts exerted a cytotoxic effect on breast cancer cells (MDA-MB-231) even at low concentrations, with an IC_{50} of 301.4 ± 10.26 μg/mL [30].

Table 4 Amount of polyphenolic compounds identified in the ethanol extract of *Melissa officinalis* leaves

No.	Polyphenolic compound	Retention time (min)	Amount (g/kg dry weight)
I	3,4-Dihydroxyphenyl lactic acid	10.04	0.50
II	3,4-DHBA	12.56	0.09
III	Caffeic acid	21.94	0.34
IV	Luteolin-7-O-glucoside	22.03	0.19
V	Rosmarinic acid glucoside	32.98	1.22
VI	Methyl caffeate	36.85	0.09
VII	Rosmarinic acid	38.11	45.51
VIII	Isolithospermic acid	41.1	3.12
IX	Methyl rosmarinate	41.69	0.26
X	Lithospermic acid	42.48	8.78
XI	Methyl isolithospermate	43.49	0.71
XII	Methyl lithospermate	44.94	1.03
Total			61.84

Our results are in agreement with the previous reported studies. The cytotoxic response of three cell lines to the three extracts is evaluated using the MTT assay. MTT assay is a well-known, rapid laboratory test and a standard colorimetric assay for measuring cellular cell growth. Our data showed that the ethanol and dichloromethane leaf extracts of *Melissa officinalis* inhibited cell proliferation in a dose-dependent manner in all three cell lines, to a greater or lesser extent, whereas the hexane extract from the leaf of *Melissa officinalis* was only effective against the LNCAP cell line. The dichloromethane and ethanol extracts expressed the highest cytotoxic activity against MCF7 cells. Of the prostate cancer cell lines, the LNCaP cell line was more sensitive to the effect of the extracts compared to PC3.

Table 5 Amount of polyphenolic compounds identified in the dichloromethane extract of *Melissa officinalis* leaves

No.	Polyphenolic compound	Retention time (min)	Amount (g/kg dry weight)
I	3,4-dihydroxyphenyl lactic acid	10.26	0.04
XIII	*o*-Tyrosol	17.31	0.14
III	Caffeic acid	19.82	0.06
XIV	Methyl hydroxyphenyl acetic acid	22.41	0.08
VI	Methyl caffeate	35.60	0.16
VII	*t*-Rosmarinic acid	36.73	1.69
XV	*c*-Rosmarinic acid	36.99	0.55
VIII	Isolithospermic acid	39.84	0.01
X	Lithospermic acid	41.26	0.07
XI	Methyl isolithospermate	42.49	0.01
XII	Methyl lithospermate	44.04	0.05
Total (g/kg)			2.84

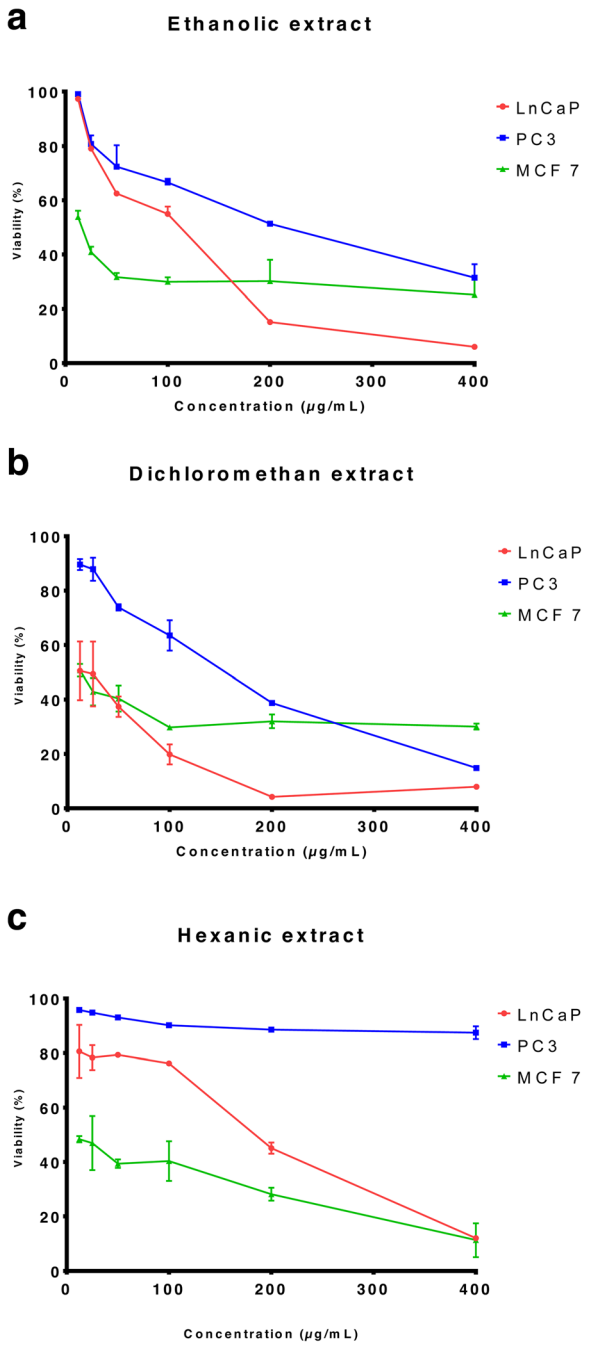

Fig. 6 Effect of *Melissa officinalis* extracts on cell proliferation of MCF7, LNCAP, and PC3 cell lines, presented as a percentage of cell viability, versus concentration of the extracts. Dichloromethane (**a**) ethanol (**b**) and *n*-hexane (**c**) extracts with increasing concentrations in the range 12.5 to 400 µg/mL for each extract. Data represent means ± SEM of experiments performed in duplicate. Untreated cells were used as negative control and mitomycin C was used as positive control

Table 6 IC_{50} values of *Melissa officinalis* extracts in four MCF7, LNCAP, and PC3 cell lines

Extracts	IC50 values (μg/mL)		
	MCF7	LNCAP	PC3
Hexane extract	ND	203.2 ± 1.118 [c]	ND
Ethanol extract	35.52 ± 1.462 ns	136.4 ± 0.814 [a]	237.82 ± 2,353 [c]
Dichloromehane extract	30.90 ± 1.462 ns	71.21 ± 1.105 [a]	173.93 ± 1,07 [b]

ND not determined
The statistical significance of the results was evaluated by the one-way ANOVA. (ns) = $p > 0.05$,
[a]$p < 0.05$
[b]$p < 0.01$ means
[c]0.001 significant difference between positif control and *Melissa Officinalis* extracts

Conclusions

In conclusion, the results of the current study indicate that dichloromethane and ethanol extracts of *Melissa officinalis* exhibit cytotoxicity against three cancer cell lines namely MCF7, LNCAP and PC3. The dichloromethane and ethanol extracts were found to be far more cytotoxic against breast cancer MCF7 cell lines as opposed to prostate cancer cell lines.

However, the cytostatic effects of the extracts, although very similar, do not correlate with the content of total polyphenolic compounds in the dichloromethane (2.84 g/kg) and ethanol extracts (61.84 mg/kg).

Abbreviations

MCF7: Michigan Cancer Foundation-7; LNCAP: Lymph node carcinoma of the prostate; PC3: Human prostate cancer cells; IC_{50}: Half maximal inhibitory concentration; GC-MS: Gas chromatography-mass spectrometry; HPLC: High performance liquid chromatography; HPLC-ESI-MS: High-performance liquid chromatography/electrospray ionization tandem mass spectrometry; DMEM: Dulbecco's modified Eagle's medium; RPMI: Roswell Park Memorial Institute *medium*; *MTT*: 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; DMSO: Dimethylsulfoxide; ANOVA: One-way analysis of variance; DCM: Dichloromethane; BSTFA: N,O-Bis(trimethylsilyl)trifluoroacetamide; MDA-MB-468: MD Anderson-Metastatic Breast-468; MDA-MB-231: MD Anderson-Metastatic Breast-231; K562: Human chronic myeloid leukemia cells; LCFA: Long chain fatty acids; UV: Ultraviolet

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Authors' contributions

K.F. and R.W.O. contributed to the research design and phytochemical studies. A.B. contributed to the evaluation and phytochemical data analysis. A.M, ElA.F, ElM.M., El.B., and B.L. contributed to the research guidance and Biological Data determinations. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors have read and approved the manuscript.

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Availability of data and materials

All data provided in the manuscript are available upon request.

Ethics approval and consent to participate

The authors declare that the work did not involve humans or animals which required ethical approval or consent to participate.

Consent for publication

Not applicable

Competing interests

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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