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Nepeta curviflora essential oil: Phytochemical composition, antioxidant, anti-proliferative and anti-migratory efficacy against cervical cancer cells, and α-glucosidase, α-amylase and porcine pancreatic lipase inhibitory activities

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ABSTRACT

The essential oils extracted from plants play an important role in medicine in addition to their huge value in the cosmetics and nutraceutical industries. In this investigation, besides the identification and quantification of the phytochemical composition of *Nepeta curviflora* Boiss essential oil, *in-vitro* antioxidant, porcine pancreatic lipase, α-glucosidase, and α-amylase inhibitory activities of the essential oil obtained by microwave ultrasonic-assisted extraction were investigated. More importantly, it was designed to assess the essential oil impact on cell migration and cell proliferation of cervical cancer cell lines. The phytochemical composition of *Nepeta curviflora* essential oil was qualitatively and quantitatively determined utilizing gas chromatography-mass spectrometry technique. Twenty compounds were identified from *Nepeta curviflora* essential oil representing 100% of total essential oil, of which 1,6-dimethyl spiro[4.5]decane (27.51%), caryophyllene oxide (20.08%), and β-caryophyllene (18.28%) were the most abundant compounds. The antioxidant activity of the essential oil was measured as 6.3 ± 0.43 µg/mL in comparison with trolox (positive control) employing the 1,1-diphenyl-2-picrylhydrazyl radical scavenging assay. The high concentrations of the caryophyllene oxide and caryophyllene thought to contribute significantly to the antioxidant of the essential oil. The obtained essential oil displayed α-glucosidase, α-amylase, and porcine pancreatic lipase inhibitory activities with half-maximal inhibitory concentration (IC_{50}) values of 26.3 ± 0.57, 45.7 ± 0.26, and 54.9 ± 0.34 µg/mL, respectively, in comparison with the positive controls orlistat and acarbose which showed IC_{50} values of 12.3 ± 0.33, 28.84 ± 1.22 and 37.15 ± 0.32 µg/mL, respectively. Moreover, anticancer activity was established exploiting wound healing and cell proliferation assays against cervical cancer cell lines. Remarkably, non-cytotoxic concentrations of *Nepeta curviflora* essential oil have significantly inhibited the migration potential of cervical cancer cells after 24 h of treatment and revealed that the oil concentration and treatment time increase the inhibitory action on cervical cancer cell viability.

1. Introduction

For centuries, phytotherapeutic products have been utilized as a prime natural source of medicines worldwide due to their effectiveness, low-cost and minimal side effects compared with the synthetic alternatives (Santini et al., 2017). Natural essential oils (EOs) extracted mainly from plant constitutes a mixture of volatile and aromatic compounds (Jaradat et al., 2016a). Nevertheless, EOs such as anise (*Pimpinella anisum* L.), caraway (*Carum carvi* L.), fennel (*Foeniculum vulgare* Mill.), clove (*Syzygium aromaticum* (L.) Merr. & L.M.Perry), cinnamon (*Cinnamomum verum* J.Presl), and many others are widely utilized from ancient times in folkloric medicine (Sharifi-Rad et al., 2017). Nowadays,

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more than 5000 tones are the annual production of natural EOs and more than 100 kinds of the valuable EOs are available commercially due to their wide range of curative potentials such as anti-inflammatory, antioxidant, antiviral, anthelmintic, antibacterial, and many other effects (Caiger, 2014; Sikka and Bartolome, 2018). In addition to their therapeutic uses, EOs are widely utilized as flavoring agents in the food industry and as odorants in perfumes and cosmetics industries (Malik, 2018).

Free radicals' reactive nitrogen and oxygen species are produced in many of the living organisms by different endogenous systems, exposure to various pathological states, or physiochemical conditions. Intake of antioxidants, free radicals scavengers, is substantial for physiological processes (Tas et al., 2018). Free radicals harm DNA, proteins, and lipids, causing various illnesses such as diabetes mellitus, neurodegenerative disorders, cancer, and cardiovascular diseases (Hecht et al., 2016). Hence, antioxidants' intake helps in slowing and/or preventing oxidative stress. Several experimental studies revealed that the presence of high levels of antioxidants could be beneficial in inhibiting several types of free radical damages that have been linked with the development of cancer (Carini et al., 2017; Patterson et al., 1997; Poprac et al., 2017).

Despite the extensive efforts and researches that have been undertaken towards the risk of obesity on health, its prevalence is still increasing rapidly. Obesity presents a global concern, not only for the physiological harm that could cause, but also due to its association with several diseases and disorders such as metabolic, endocrine, and cardiovascular diseases (Seiler et al., 2018).

Diabetes mellitus is considered to be the most widespread and life-threatening disease, affecting millions of people worldwide. The Global Burden of Disease (GBD) estimated a 30.6% rise in the prevalence of diabetes from 2005 to 2015. International Diabetes Federation estimated that around 360 million people with diabetes, and this number could double by 2030 (Mitra et al., 2012). It has been reported that obesity is considered to be a major leading risk factor of diabetes type 2 (Olokoba et al., 2012).

Cancer is considered to be one of the most life-threatening diseases in the last two decades. The main feature of cancer is its ability in the cloning of tumors from single-cell that begin to proliferate quickly and abnormally (Cooper, 2000). This abnormal proliferation process can be retarded by activities such as apoptosis, cytotoxicity, and anti-proliferative activity (Bender and Martinou, 2013).

In nature, about 300 plant species represented the *Nepeta* genus that belongs to the Lamiaceae family. These species are broadly utilized in folk medicine in many regions of Asia, Europe, and Africa (Hadi et al., 2017). Their decoctions and infusions are widely used as pain relief, and for medications of intestinal worms, fever, upper respiratory system infections, joints inflammations, cancer, and dermatitis (Amira et al., 2012; Kaska et al., 2018; Shahri et al., 2016; Skorić et al., 2017).

Nepeta curviflora Boiss, commonly known as Syrian Catmint, is an essential oil-bearing perennial herbaceous plant growing up to 60 cm with bluish-violet flowers. This plant germinates wildly in the mountains of Palestine, Jordan, Syria, Lebanon, and Turkey (Barhoumi et al., 2017).

This investigation was designed to explore the chemical composition of *N. curviflora* EO, collected from the Birzeit mountains in Palestine, quantitatively and qualitatively using GC–MS as well as to evaluate it's in *vitro* anti-oxidative, antilipase, antiamylase, and antiglucosidase activities. Additionally, it was aimed to evaluate the *N. curviflora* EO effects on cervical cancer cell migration and growth. To the best of authors' knowledge, the chemical composition and biological activity of *N. curviflora* EO vegetating in Palestine have never been reported.

2. Material and methods

2.1. Equipment

Microwave ultrasonic extractor (CW-2000, Hong Kong, China), laminar flow (MRC, BBS12HGs, Israel), electronic balance (Wagl, AS 220/C/2, Radwag, Poland), inverted biological microscope ((LaboMed-ARCO Med TCM-400 microscope, USA), 24-well plates and 96-well plates (Greiner bio-one, North America), shaking laboratory water bath (Lab Tech, BPXOP1001040, Namyangju, South Korea), CO_2 incubator (ESCO, 2012-74317, Singapore), micropipettes (Finnpipette, Finland), horizontal microplate reader (Unilab, RTC 6000, USA), ELISA plate reader (BioRad, 680 XR, Japan) and spectrophotometer (Jenway, UV/Vis Spectrophotometer 6505, UK) were used to complete this investigation.

2.2. Chemicals

Dimethyl sulfoxide (DMSO) (Riedel-de-haen, Q4199, Germany), 2,2-diphenyl-1-picrylhydrazyl

(DPPH) (Sigma-Aldrich, D9132-1G, Germany), 3,5-dinitro salicylic acid (Sigma-Aldrich, USA),

Methanol (Loba Chemie, India), trolox ((s)-(−)-6 hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) (Sigma-Aldrich, 391921-1G, Denmark), RPMI-1640 medium (Roswell Park Memorial Institute-1640 medium) (Sigma-Aldrich, R0883, UK), Tris−HCl buffer (SDFCL, India), Elisa kit (Brookhaven, 280173, NewYork, USA). CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay (Promega Corporation, Madison, USA, porcine pancreatic lipase (Sigma-Aldrich, USA), orlistat (Sigma-Aldrich, Germany), *p*-nitrophenyl butyrate (Sigma-Aldrich, Germany), porcine pancreatic α-amylase (Sigma-Aldrich, India), acarbose (Sigma, USA), PBS (phosphate buffer saline) (Sigma-Aldrich, 79383, Germany), *p*-nitrophenyl α-D-galactopyranoside (Sigma-Aldrich, USA), and MTS (Manothermosonication) (Spanish, 9200686, Spain).

2.3. Plant collection and preparing

The leaves and flowers were collected from the Birzeit mountains in Palestine at the end of May 2019. The botanical identification was approved at the Pharmacognosy Laboratory, An-Najah National University by the pharmacognosist Dr. Nidal Jaradat. The dried herbarium was kept at the same Laboratory within the voucher specimen number of Pharm-PCT-1633. The fresh leaves and flowers were thoroughly washed using distilled water then left to dry in the shade at humidity (55 ± 5 RH) and temperature ($25 \pm 2\,^{\circ}C$) for two weeks. The dried parts were grinded utilizing a mechanical blender and stored in paper bags for further isolation process.

2.4. Extraction of the essential oil

The *N. curviflora* EO was obtained utilizing the microwave ultrasonic apparatus as described previously by Jaradat et al. (Jaradat et al., 2016a). One-liter round-bottom flask containing 50 g of the dried plant powder and 500 mL of distilled water was placed in the microwave-ultrasonic apparatus. The flask was attached to the clevenger apparatus which was placed in the same apparatus. The microwave extractor apparatus power was adjusted at 1000 W for 15 min at 100 °C. The extracted EO was dried using calcium chloride and stored at 2−8 °C. The isolated EOs yield average was 1.7% from the dried parts.

2.5. Identification of the phytochemical composition of the essential oil by gas chromatography-mass spectrometry (GC–MS)

The separation and identification of *N. curviflora* EO components were achieved employing the Perkin Elmer Elite-5-MS fused-silica

capillary column (30 m × 0.25 mm, film thickness 0.25 μm), where Helium was used as a carrier gas at a standard flow rate of 1.1 mL/min. The temperature of the injector was adjusted at 250 °C with an initial temperature 50 °C, initial hold 5 min, and ramp 4.0 °C/min to 280 °C. The total running time was 62.50 min and the solvent delay was from 0 to 4.0 min. MS scan time was from 4 to 62.5 min, covering the mass range from 50.00 to 300.00 *m/z*. The chemical constituents of *N. curviflora* EO were identified by comparing their mass spectra with the reference spectra from the MS Data Centre of the National Institute of Standards and Technology, and by matching their kovats and retention indices with values described in the literature (Vinaixa et al., 2016; Wei et al., 2014). The EOs kovats and retention indices values were compared with 20% of HPLC grade of reference EOs purchased from Sigma-Aldrich (Germany) (Serfling et al., 2007).

2.6. Free radical scavenging assay

The free 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging approach was adopted for determining the antioxidant potential of *N. curviflora* EO (Jaradat and Abualhasan, 2015). A solution of the EO in methanol (1 mg/mL) was prepared followed by the preparation of a solution of trolox in methanol (1 mg/mL). Then a dilution series from the stock solutions, giving six serial dilutions at concentrations of 2, 5, 10, 20, 50, and 100 μg/mL were produced. One mL of each EO dilutions was mixed with 1 mL of 0.002 g/mL methanolic DPPH solution. Methanol (1 mL) was added to give a final working volume of 3 mL. The DPPH solution was freshly prepared due to its sensitivity to light. The blank control of the series concentrations was prepared by dissolving DPPH in methanol in 1:2 ratios in the absence of the EO. All solutions were incubated at 25 °C under the exclusion of light for 30 min. The absorbance was measured using a spectrophotometer at a wavelength of 517 nm.

The equation below was used to determine DPPH inhibition percentage for *N. curviflora* EO, with trolox:

$$\text{DPPH inhibition}\% = (A_w - A_t)/A_w \times 100\%$$

where A_w is the working solution recorded absorbance, and A_t is the established trial solution recorded absorbance.

2.7. Porcine pancreatic lipase inhibition assay

The porcine pancreatic lipase inhibition was assessed employing a previously reported procedure (Jaradat et al., 2017). A stock solution of *N. curviflora* EO in 10% dimethyl sulfoxide (DMSO) (500 μg/mL) was prepared, of which a dilution series of five concentrations of 50, 100, 200, 300, and 400 μg/mL were produced. A freshly stock solution of porcine pancreatic lipase in the Tris−HCl buffer (1 mg/mL) and a solution of 20.9 mg of *p*-nitrophenyl butyrate (PNPB) in 2 mL of acetonitrile were prepared. For each working solution, 0.1 mL of porcine pancreatic lipase (1 mg/mL) was mixed with 0.2 mL of *N. curviflora* EO from each of the dilution series, followed by addition of Tris−HCl to bring the final volume of the working solutions to 1 mL. After 15 min incubation at 37 °C, a solution of *p*-nitrophenyl butyrate in acetonitrile (0.1 mL) was added to each test-tube and the resulting mixture was incubated at 37 °C for an additional 30 min. Pancreatic lipase activity was assessed by measuring the hydrolysis of PNPB into *p*-nitrophenolate using a UV spectrophotometer at 410 nm. The same experiment was repeated using orlistat (positive control). Percentage lipase inhibition by plant EO was calculated using the following equation:

$$\text{Lipase inhibition}\% = (A_B - A_{ts})/A_B \times 100\%$$

where A_B is the recorded absorbance of the blank solution and A_{ts} is the recorded absorbance of the tested sample solution.

2.8. α-Amylase inhibition assay

The α-amylase inhibitory efficiency of *N. curviflora* EO was evaluated using a standard method with minor modifications (Jaradat and Al-Maharik, 2019). Essential oil (100 mg) was dissolved in a few milliliters of 10% DMSO, and then 0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl buffer solution (pH 6.9) was added to bring the volume to 100 mL offering a stock solution having a concentration of 1 mg/mL, of which the following dilutions 10, 50, 70, 100 and 500 μg/mL were prepared using 10% DMSO as the diluent.

A porcine pancreatic α-amylase (0.2 mL of 2 units/mL) was mixed with *N. curviflora* EO (0.2 mL). After 10 min incubation at 30 °C, a freshly prepared 1% starch solution in water (0.2 mL) was added, the tubes were incubated for at least 3 min. At this point, the reaction was quenched by adding 3,5-dinitro salicylic acid (DNSA), and the mixture was diluted with distilled water (5 mL), before being heated at 90 °C in a water bath for 10 min. The mixture was then cooled down to room temperature, and the absorbance was measured at 540 nm. The blank control was prepared using the same quantities described above by replacing *N. curviflora* EO with buffer (0.2 mL). Acarbose was used as a standard reference following the procedure described above. The α-amylase inhibitory activity was calculated using the following equation:

$$\text{α-Amylase inhibition}\% = (A_B - A_T)/A_B \times 100\%$$

where A_B is the absorbance of the blank sample and A_T = absorbance of the test sample.

2.9. α-Glucosidase inhibitory assay

The α-glucosidase inhibitory action of *N. curviflora* EO was evaluated according to the standard protocol with minor modifications (Hawash et al., 2019). A dilution series of *N. curviflora* EO was prepared, to yield concentrations of 100, 200, 300, 400, and 500 μg/mL. The reaction mixtures contained α-glucosidase solution (0.1 mL of 1 U/mL) were mixed with 0.2 mL of the EO dilution and 0.5 mL of 100 mM phosphate buffer (pH 6.8). The mixtures were incubated at 37 °C for 15 min, then 5 mM *p*-nitrophenyl α-D-galactopyranoside (PNPG) was added to each of the reaction mixtures and incubated at 37 °C for additional 20 min.

The reactions were terminated by adding 0.1 M Na_2CO_3 and the absorbance was recorded at a wavelength of 405 nm for the EO sample. Acarbose was used as a positive control at the same concentrations as the plant EO. The inhibition percentages were calculated according to the equation below:

$$\text{α-Glucosidase inhibition}\% = (A_B - A_s/A_B) \times 100\%$$

where A_B is the absorbance without enzyme inhibitor and A_S = absorbance in the presence of the enzyme inhibitor.

2.10. Cervical cancer cells culture

HeLa cells are human epithelial eternized cell lines of the uterine cervix carcinoma mutated by human papillomavirus 18 (HPV18). The used media RPMI-1640 was complemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin, and 1% L-glutamine to maintain cell health. The cells were incubated with or without treatment at 37 °C and in a humidified atmosphere of 5 percent CO_2.

2.11. Cell proliferation (growth) assay

HeLa cells were cultured in RPMI-1640 media with 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin/streptomycin antibiotics. Cells were cultivated at 2.6×10^4 cells/well in a 96-well plate in a humid atmosphere with 5% $CO_2$and at 37 °C. Thereafter, the cells were incubated with 0.078, 0.156, 0.3125, 0.625, 1.25, and 2.5 mg/mL of

N. curviflora EO for 24 h and 48 h. Cell viability was assessed by CellTiter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the instructions of the manufacturer (Promega Corporation, Madison, WI). Finally, 20 µL of MTS solution per 100 µL of media was added to each well and the plate was incubated at 37 °C for 2 h. The absorbance was recorded at 490 nm (Amer et al., 2019).

2.12. Wound healing assay (cell migration test)

HeLa cells were seeded in 24-well plates until confluency was achieved. The confluent monolayer of cells was scratched vertically to form a wound with the tip of a sterile plastic pipette along the diameter of each well. Three wells for each condition were considered per experiment. Plate wells have been washed with PBS and incubated in RPMI-1640 complete media at 37 °C in the presence of vehicle or the concentrations of *N. curviflora* EO mentioned above (Abdallah et al., 2019).

2.13. Images quantification

The wound length crossing the well diameter was photographed. Three images were taken per well, and three wells were considered for each condition. Altogether, nine reads for each condition were considered per experiment. Wound width was imaged using an inverted microscope (LaboMed- ARCO Med TCM-400 microscope, https://www.laboamerica.com/products/life-materials-sciences/tcm-400#specifications) at a total magnification of 40X (objective lens 4× * 10×/22 mm eyepiece). The area of the wound was measured at 0 h and 24 h using ImageJ software including MRI Wound Healing Tool (http://dev.mri.cnrs.fr/projects/imagej-macros/wiki/Wound_Healing_Tool). Wound area invaded by cells after 24 h was calculated as per the following: (area of the wound at 0 h – area of the wound at 24 h). Eventually, the migration area for each treated condition was represented as a percentage relative to control values.

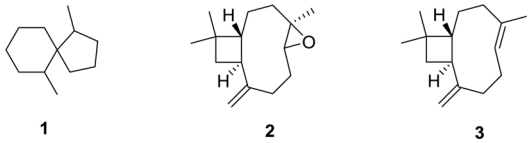
2.14. Data analysis

Data has been expressed as means ± SEM. Statistical analysis was conducted using the Student *t*-test. Differences considered being significant at $p \leq 0.05$. Calculations of the inhibitory concentration 50% (IC_{50}) were obtained from the Prism dose-response curve (Prism Graph pad, version 7.0 of prism for Windows (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. GC–MS characterization of N. curviflora essential oil

The chemical constituent of *N. curviflora* EO from Palestine was quantitatively and qualitatively characterized by utilizing the GC–MS technique. Twenty compounds were identified amounting to 100% of total EO. 1,6-Dimethyl spiro[4.5]decane **1** (27.5%), caryophyllene oxide **2** (20.08%), and *β*-caryophyllene **3** (18.28%) were discovered to be the most abundant components of *N. curviflora* EO (Table 1). It is quite interesting to point out that caryophyllene oxide **2**, and *β*-caryophyllene **3**, which possess high potential, were found in higher concentrations in EO *N. curviflora* growing in Palestine than in the studied plant vegetating in Lebanon and Jordan.



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The phytochemical composition, retention index (RI) and retention time (RT) with their concentration (%) are depicted in Table 1 and the GC–MS chromatogram (Fig. 1).

Table 1
The chemical composition of *Nepeta curviflora* essential oil.

no	Compounds	MW	RI	Identification	% Area
	trans-Carane	138.25	808	MS, RI	0.07
	p-Mentha-3,8-diene	136.23	772	MS, RI	1.58
	1,3,4-Trimethyl-3-cyclohexenyl-1-carboxaldehyde	152.23	803	MS, RI	0.62
	Limona ketone	138.21	731	MS, RI	1.58
	1,6-Dimethyl spiro[4.5]decane	166.3	922	MS, RI	27.51
	Decahydro-3a-methyl-6-methylene-1-(1-methylethyl) cyclobuta[1,2:3,4] dicyclopentadiene	230.39	787	MS, RI	1.97
	Nepetalactone	166.22	840	MS, RI	5.38
	cis,trans-1,9-Dimethyl spiro [4.5]decane	166.3	875	MS, RI	1.64
	β-Caryophyllene	204.36	924	MS, RI	18.28
	β-Farnesene	204.35	792	MS, RI	6.12
	α-Humulene	204.35	904	MS, RI	1.19
	Aromadendrene	204.35	917	MS, RI	0.50
	β-Cubebene	204.35	883	MS, RI	2.96
	Humulen-(v1)	204.35	916	MS, RI	0.17
	γ-Elemene	204.35	837	MS, RI	1.81
	γ-Caryophyllene	204.35	830	MS, RI	4.95
	β-Sesquiphellandrene	204.35	843	MS, RI	2.26
	Argeol	220.35	734	MS, RI	1.07
	4-Methylene-2,8,8-trimethyl-2-vinylbicyclo[5.2.0]nonane	204.35	799	MS, RI	0.27
	Caryophyllene oxide	220.35	732	MS, RI	20.08
	Total (%)				100.00
Grouped components			%		
1.	Sesqueterene hydrocarbon		38.49		
2.	Alcoholic sesquiterpenoid		1.07		
3.	Oxide sesquiterpenoid		20.08		
4.	Monoterpene hydrocarbon		1.65		
5.	Aldehydic monoterpenoid		0.62		
6.	Ketonic monoterpenoid		1.97		
7.	Monoterpenoid lactone		5.38		
8.	Spiroalkane		29.14		
9.	Ketone		1.58		
Total identified groups (%)			100.00		

MW: molecular weight (g/mol); RI: retention index.

3.2. Antioxidant potential

The anti-oxidant potential of the *N. curviflora* EO was analyzed using the DPPH colorimetric assay. The radical scavenging activity of *N. curviflora* EO and trolox were articulated as a percentage of DPPH inhibition. The DPPH radical scavenging activity of the EO was assessed as 6.3 ± 0.43 µg/mL, two-fold weaker than that displayed by trolox (IC_{50} = 6.3 ± 0.43 µg/mL) as indicated in Table 2 and Fig. 2.

3.3. Porcine pancreatic lipase inhibitory activity

In this assay, the anti-obesity activity of *N. curviflora* EO was compared with that of the orlistat, a potent lipase inhibitory agent. The plant EO and orlistat exhibited anti-obesity activity with IC_{50} values of 26.3 ± 0.57 and 12.3 ± 0.33 µg/mL, respectively, as shown in Table 3 and Fig. 3.

3.4. α-Amylase activity

The lipase-catalyzed hydrolysis of *p*-nitrophenyl butyrate to the chromophore (*p*-nitrophenol) procedure was utilized to determine the inhibitory activity of *N. curviflora* EO on the porcine pancreatic *α*-amylase enzyme. The assay was compared with acarbose, a strong *α*-amylase inhibitory agent, and the IC_{50} values were calculated for *N. curviflora* EO and acarbose (Table 4 and Fig. 4). The IC_{50} of the EO and

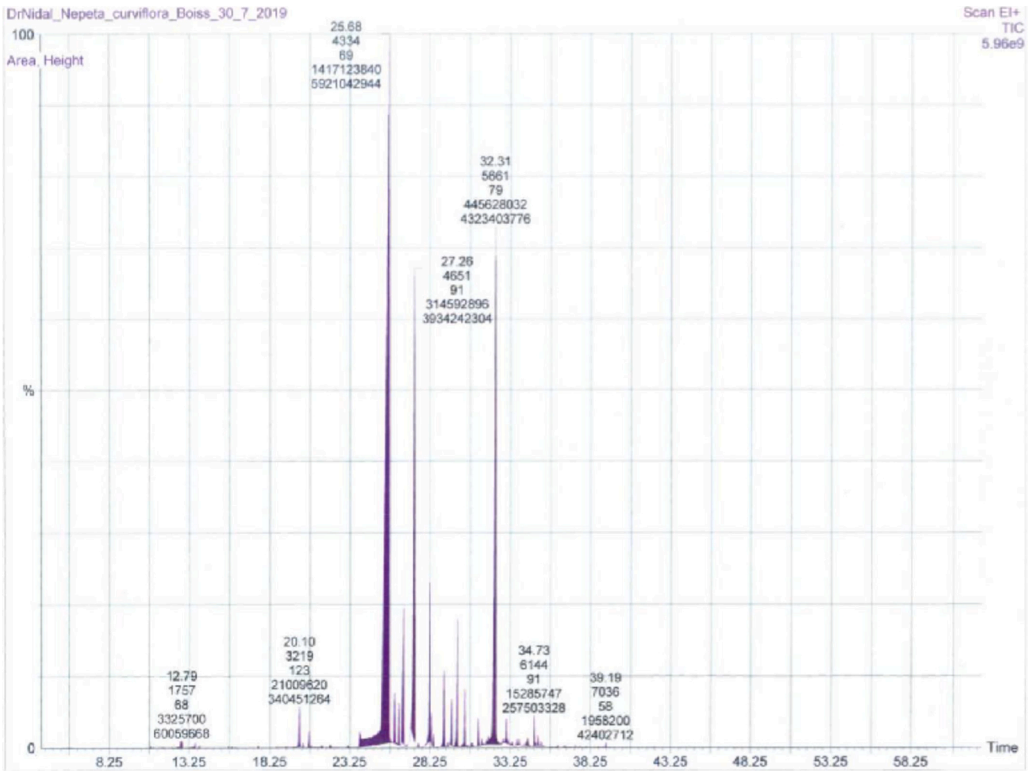

Fig. 1. *Nepeta curviflora* essential oil Gas chromatography-mass spectrometry chromatogram.

Table 2
The inhibitory activity of *Nepeta curviflora* essential oil and trolox against DPPH.

Concentrations (μg/mL)	Trolox	*Nepeta curviflora* essential oil
0	0 ± 0.00	0 ± 0.00
2	53.41 ± 2.26	52.24 ± 0.00
5	61.52 ± 0.33	52.24 ± 0.00
10	91.42 ± 0.33	53.46 ± 1.62
20	97.28 ± 0.33	77.02 ± 0.31
50	97.28 ± 0.33	77.02 ± 0.33
100	97.55 ± 0.68	81.58 ± 0.3
IC_{50}	3.1 ± 0.92	6.3 ± 0.43

Values represent the mean ± SD ($n = 3$/group).
IC_{50}=half maximal inhibitory concentration, DPPH = 1,1-diphenyl-2-picrylhydrazyl.

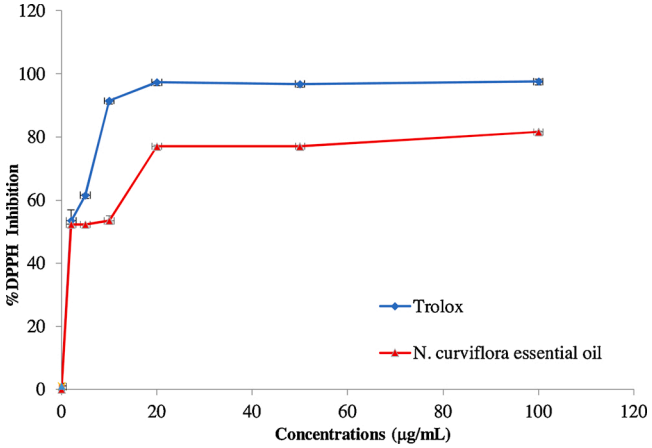

Fig. 2. Inhibition% of DPPH (1,1-diphenyl-2-picrylhydrazyl) by trolox and *Nepeta curviflora* essential oil.

Table 3
The inhibitory activity of *Nepeta curviflora* essential oil and orlistat against porcine pancreatic lipase.

Concentrations (μg/mL)	Orlistat	*Nepeta curviflora* essential oil
0	0 ± 0.00	0 ± 0.00
50	91.05 ± 0.77	64.52 ± 0.47
100	93.1 ± 0.42	64.69 ± 0.72
200	94.3 ± 0.42	64.69 ± 0.72
300	97.4 ± 0.12	97.96 ± 0.47
400	97.5 ± 0.00	98.95 ± 0.49
IC_{50}	12.3 ± 0.33	26.3 ± 0.57

Values represent the mean ± SD (n = 3/group).
IC_{50}=half maximal inhibitory concentration.

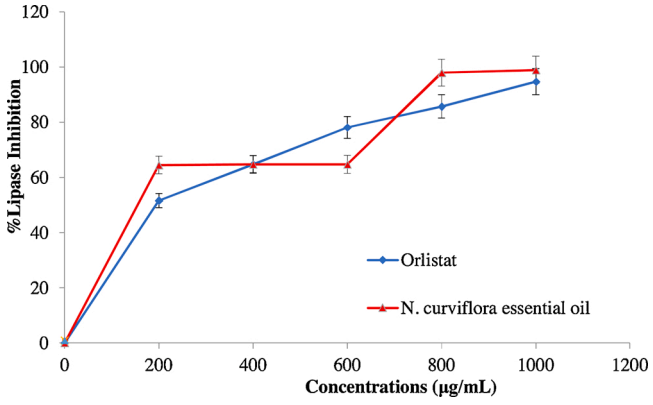

Fig. 3. Inhibition percentage of lipase by *Nepeta curviflora* essential oil and orlistat.

Table 4
α-Amylase inhibition of *Nepeta curviflora* essential oil compared with acarbose.

Concentrations (μg/mL)	Acarbose	*Nepeta curviflora* essential oil
0	0 ± 0.00	0 ± 0.00
10	53.22 ± 1.2	20.3 ± 0.007
50	54.91 ± 0.58	63.53 ± 0.21
70	66.1 ± 1.34	63.53 ± 0.21
100	66.1 ± 1.62	65.21 ± 0.41
500	72.54 ± 1.37	65.8 ± 0.45
IC_{50}	28.84 ± 1.22	45.7 ± 0.26

Values represent the mean ± SD (n = 3/group).
IC_{50}=half maximal inhibitory concentration.

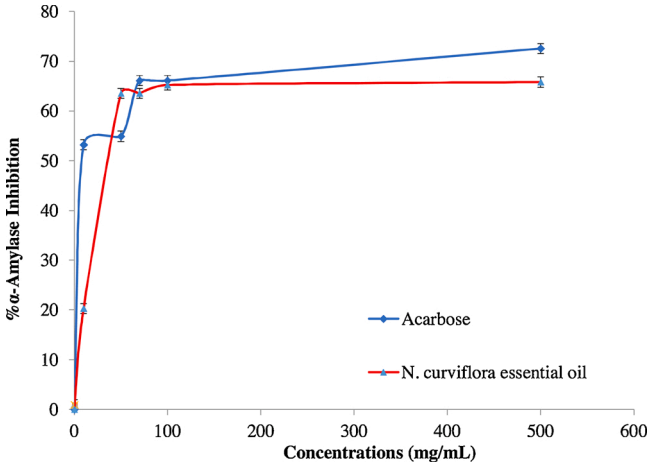

Fig. 4. α-Amylase inhibition of *Nepeta curviflora* essential oil compared with acarbose.

the positive control were measured as 45.7 ± 0.26 and 28.84 ± 1.22 μg/mL, respectively.

3.5. α-Glucosidase inhibitory activity

Results for α-glucosidase were compared with those for acarbose, a strong α-glucosidase enzyme inhibitory agent, and the IC_{50} doses were calculated for *N. curviflora* EO (Table 5 and Fig. 5). The IC_{50} of the EO and the positive control were found to be 54.9 ± 0.34 and 37.15 ± 0.32 μg/mL, respectively.

3.6. N. curviflora EO inhibitory effect on the migration of cervical cancer cells

It is a fact that the majority of cancer deaths arise due to the tumors that metastasize distally to form secondary tumors. The metastasis process depends on the spread of cancer cells to tissues. The current investigation studied whether *N. curviflora* EO affects the cell migration

Table 5
α-Glucosidase inhibition of *Nepeta curviflora* essential oil compared with acarbose.

Concentrations (μg/mL)	Acarbose	*Nepeta curviflora* essential oil
0	0 ± 0.00	0 ± 0.00
100	65.81 ± 0.46	38.33 ± 0.73
200	67.76 ± 0.34	55.51 ± 0.22
300	73.34 ± 0.45	81.76 ± 0.11
400	85.39 ± 0.36	81.76 ± 0.01
500	92.28 ± 0.11	92.72 ± 0.72
IC_{50}	37.15 ± 0.32	54.9 ± 0.34

Values represent the mean ± SD (n = 3/group).
IC_{50}=half maximal inhibitory concentration.

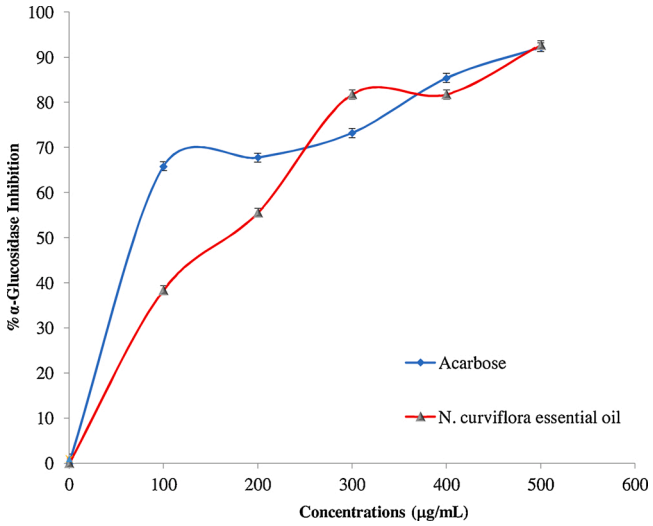

Fig. 5. α-Glucosidase inhibition of *Nepeta curviflora* essential oil and acarbose.

of cervical cancer cells. Thus, the effect of *N. curviflora* EO on the migration of HeLa cells using wound assay at concentrations of 78, 156, and 312 μg/mL after 24 h was assessed. The applied concentrations proved to be non-cytotoxic to HeLa cells utilizing MTS assay (Fig. 6A). Significant inhibition of the migration of HeLa cells at 78 μg/mL and 156 μg/mL concentrations compared with non-treated conditions or control was observed (Fig. 6B). The results strongly suggest that *N. curviflora* EO has a significant migration inhibitory effect on HeLa cells.

3.7. N. curviflora EO inhibitory effect on cervical carcinoma growth

The current investigation explored whether *N. curviflora* EO has a cytotoxic effect on cervical cancer cells. The effect of 0.078, 0.156, 0.3125, 0.625, 1.25 and 2.5 mg/mL of *N. curviflora* EO at 24 h (Fig. 7A) and 48 h (Fig. 7B) compared with non-treated cells was assessed. The results revealed that 2.5, 1.25, 0.625 mg/mL concentrations of *N. curviflora* EO have an inhibitory potential on cell viability after 24 h and 48 h of treatment. It was found that *N. curviflora* EO inhibited the HeLa cancer cell growth with IC_{50} values of 0.7469 and 0.4531 mg/mL after 24 h and 48 h of treatment, respectively. Those results together suggest an inhibitory effect of *N. curviflora* EO on cervical carcinoma viability.

4. Discussion

Twenty compounds were characterized representing 100% of the total *N. curviflora* EO, of which 1,6-dimethyl spiro[4.5]decane (27.51%), caryophyllene oxide (20.08%), and β-caryophyllene (18.28%) were the major phytochemical components. The identified compounds could be classified into eight phytochemical groups, of which sesquiterpene hydrocarbon was found to be the main group, representing 38.49% of the total EO composition. β-caryophyllene (18.28%), β-farnesene (6.2%), and γ-caryophyllene (4.95%) were the main major compounds in sesquiterpene group. Oxygenated sesquiterpene group, represented only by caryophyllene oxide which accounts for 20.08% of the total EO. Spiroalkane group represents the second major set of compounds detected with 29.14% of the total EO, of which 1,6-dimethylspiro[4.5] decane (27.51%) was the most abundant compound of the total EO. Hydrocarbon monoterpenes and oxygenated monoterpenoids (aldehyde, ketone, and lactone) amounted to 9.62% of the total oil, of which nepetalactone was the major accounting for 5.38% of the EO.

The chemical composition of *N. curviflora* EO collected from Lebanese and Jordanian mountains has been previously studied. Quantitative and qualitative variations between the current study and the

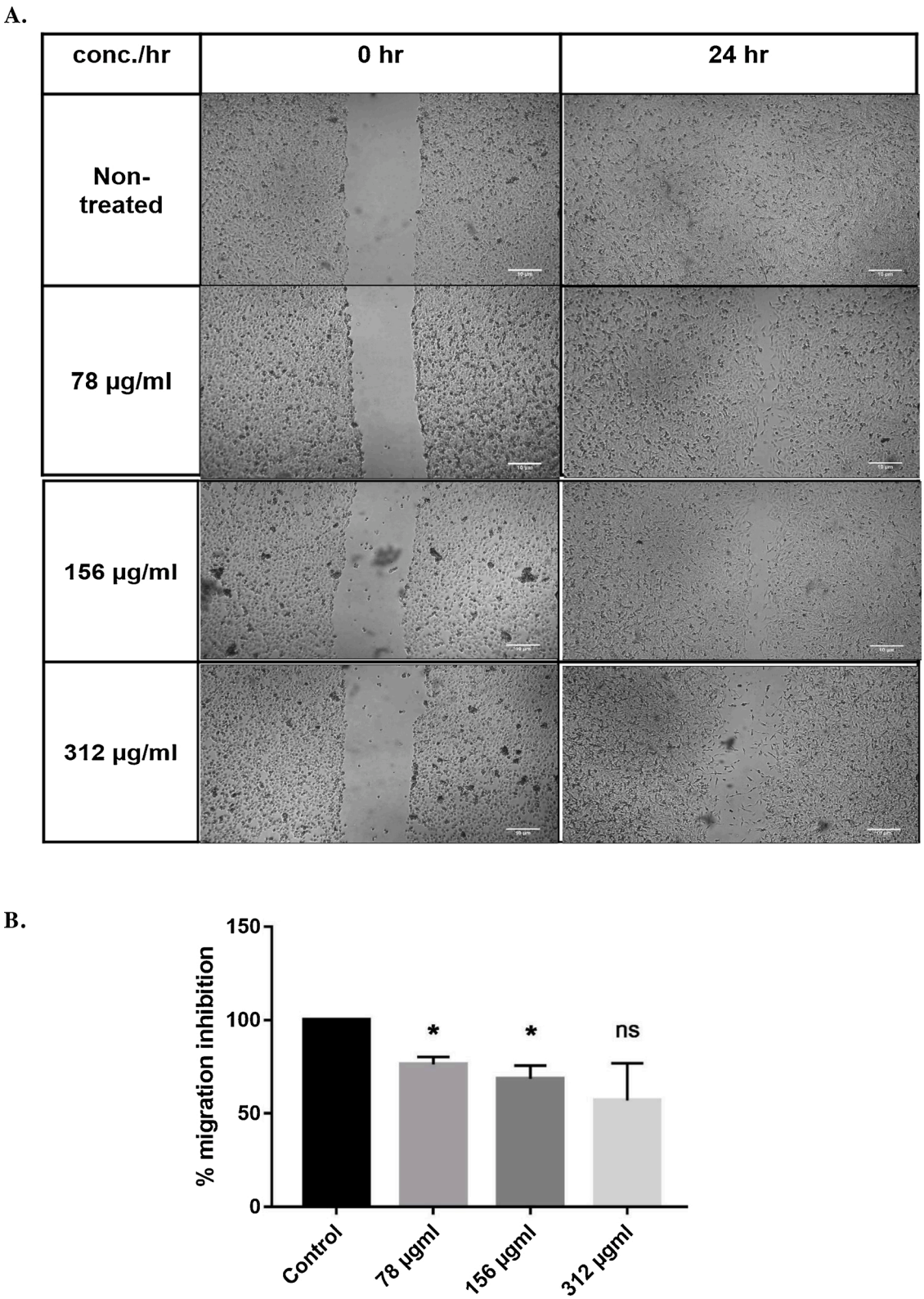

Fig. 6. *Nepeta curviflora* essential oil inhibitory effect on cervical carcinoma migration.
(**A**) Confluent HeLa cells incubated with or without 78, 156, and 312 µg/mL concentrations of *Nepeta curviflora* EO at the start or after 24 h. An inverted microscope was employed to take images of the wound at the start and after 24 h. Conc. stands for concentration. Scale bar equals 10 µm.
(**B**) Quantification of the cells' migrated area with 78, 156, and 312 µg/mL of *Nepeta curviflora* essential oil after 24 h compared with non-treated cells. Control refers to non-treated cells. Values in each column represent the percentage of inhibition of migration compared with the control condition. Data were reported in SEM; $n = 3$. *ns* = non-significant. *($p < 0.05$) indicates a statistical difference compared with control at each time point (Unpaired *t*-test).

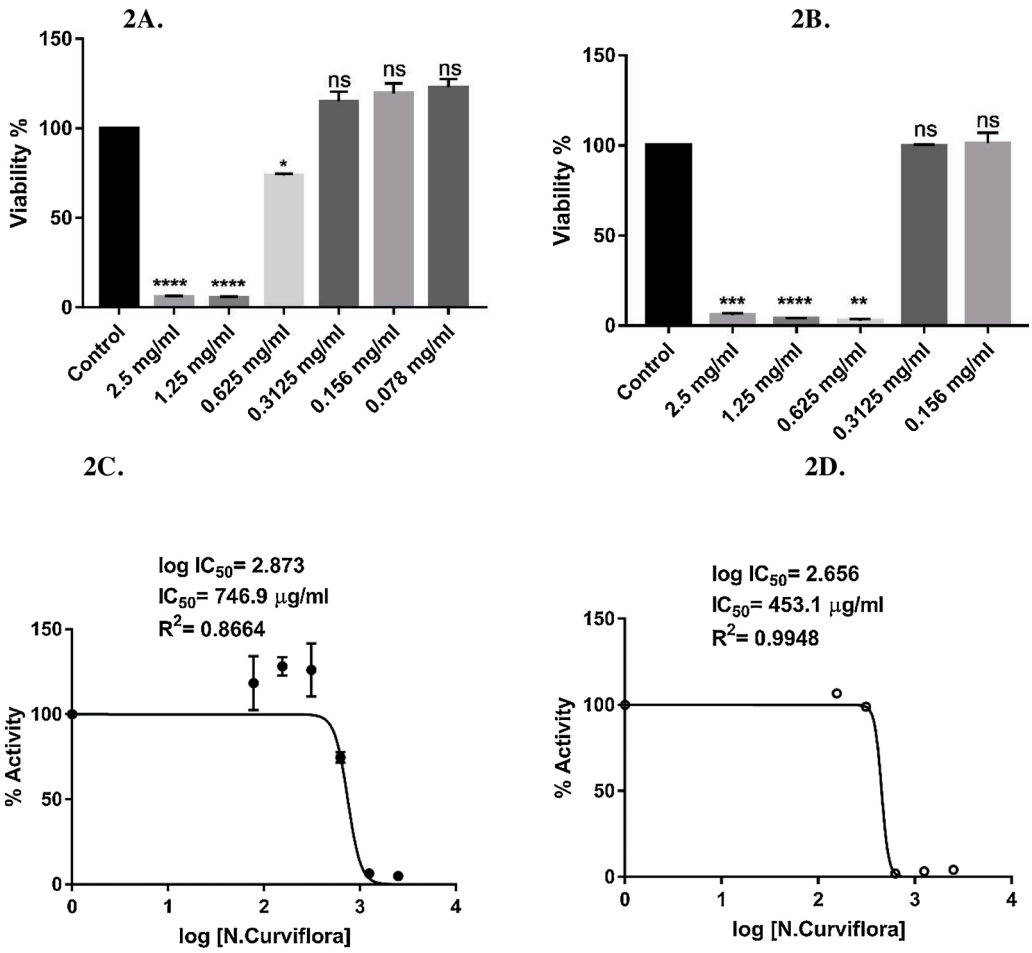

Fig. 7. *Nepeta curviflora* essential oil inhibitory effect on cervical carcinoma growth.
Quantification of cell viability of HeLa cells treated with 0.078, 0.156, 0.3125, 0.625, 1.25, and 2.5 mg/mL of *Nepeta curviflora* essential oil after 24 h (A) and 48 h (B) and compared with control (non-treated cells). Values in each column represent the percentage of inhibition relative to control. Dose-response curves showing the percentage of inhibitory activity of *Nepeta curviflora* essential oil on the viability of HeLa cells after 24 h **(C)**, and 48 h **(D)** of incubation with 0.078, 0.156, 0.3125, 0.625, 1.25, and 2.5 mg/mL of *Nepeta curviflora* essential oil. Data has been reported in SEM; $n = 3$. *ns* = non-significant. *($p < 0.05$), **($p < 0.01$), ***($p < 0.001$), ****($p < 0.0001$), indicates statistical difference relative to control at every time point (Unpaired *t*-test).

previous studies were observed. The chemical constituents of the EOs of *N. curviflora* collected from Lebanon and Jordan are reported in Table 6.

Comparing the findings of the four studies from Lebanon, Jordan and the current investigation revealed explicitly that there are qualitative and quantitative variations. These variations could be partially attributed to the extract techniques used. The current investigation used the mild microwave ultrasonic apparatus for the extraction of the EO, while the previously conducted studies used the hydrodistillation method that requires reflux for a long time (≥3 h), that can have an impact on the chemical composition of the EO. Besides, leaves and flowers *N. curviflora* were used in the current study, while the previously conducted studies used either the leaves, flowers, and stems or the flowering tops, leaves, and seeds. The qualitative and quantitative plant chemical composition depends on the parts used, extraction method, location, and the collection period. The *N. curviflora* used in the previous studies was collected in April, where the seeds were not completely formed. In this study, the plant was collected on the 22 of May, where the seeds were completely formed.

Numerous phytochemicals such as polyphenols, anthocyanins, flavonoids, carotenes, tocopherol, and ascorbic acid exhibited potential antioxidant activity (Guo et al., 2017). The pharmacodynamic effect of these molecules depends mostly on the inhibition of the DNA oxidation process which in turn prevents the oxidation of lipid and consequently diminishing the risk of numerous cancer and cardiovascular diseases (Reis et al., 2016). In nutraceutical and pharmaceutical sciences, the antioxidant potential has become one of the renowned standards in many kinds of industries, especially for the manufacturing of food preservatives, nutraceutical supplements, and free radical scavenging herbal medicines (Ndhlala et al., 2010).

The current study revealed that *N. curviflora* EO possesses a powerful antioxidant activity with an IC_{50} value of 6.3 ± 0.43 µg/mL, which is half of that of the standard antioxidant compound trolox ($IC_{50} = 3.1 \pm 0.92$ µg/mL), which is in agreement with the data stated by Al-Qudah (Al-Qudah, 2016). The *N. curviflora* EO from Jordan exhibited significant antioxidant potential with an IC_{50} dose of 0.30 ± 7.07 µg/mL compared with that of ascorbic acid ($IC_{50} = 7.22 \times 10^{-5} \pm 2.30 \times 10^{-6}$ µg/mL (Al-Qudah, 2016). This significant antioxidant potential could be attributed to the high occurrence of antioxidative compounds in the EO such as caryophyllene, caryophyllene oxide, and nepetalactone and *β*-farnesene (Fidyt et al., 2016; Shafaghat and Oji, 2010).

Obesity is a costly and long-term metabolic disorder that is hardly curable, where its prevalence rates are increasing in throughout most of the developed countries (Buskens et al., 2019). Inhibition the absorption of dietary fat was documented to be one of the appropriate methods in obesity treatment. In fact, the administration of a potent antilipase drug such as orlistat which has proven to be effective against overweight and obesity (Jaradat et al., 2016b; Kushner, 2019; Kwon et al., 2003).

Actually, the EO of *N. curviflora* showed strong antilipase inhibition activity with an IC_{50} value of 26.3 ± 0.57 µg/mL, where the enzyme activity was conducted utilizing PNPB as a substrate. The obtained IC_{50} values were compared with that of orlistat ($IC_{50} = 12.3 \pm 0.33$ µg/mL). Roh and Jung reported similar IC_{50} of porcine pancreatic lipase $37.3 \pm 2.5\%$ for a hydroethanolic extract of *Nepeta japonica* (Roh and Jung, 2012). Salameh et al. reported that the EO of *Nepeta serpyllifolia* (*Micromeria fruticosa serpyllifolia*) displayed antilipase activity with an IC_{50} value of 39.81 µg/mL compared with that of orlistat ($IC_{50} = 43.64$ µg/mL) (Salameh et al., 2018).

De facto, over thousands of years herbal products have been widely

Table 6
Chemical compositions of *Nepeta curviflora* essential oil growing in Lebanon and Jordan.

The parts used	Location	Hydro-distillation time	EOs chemical compositions	References
Aerial parts included flowering tops, seeds, and leaves	Lebanon	3.5 h	2-Isopropyl-5-methyl-3-cyclohexen-1-one, (-)-spathulenol, *cis*-*Z*-*α*-bisabolene epoxide, widdrol, (*E,Z*)-5,7-dodecadiene, dihydro-nepetalactone and 4-propyl-cyclohexene with 12.51, 11.73, 8.07, 7.0, 6.93, 5.57 and 5.43%, respectively.	(Musso et al., 2017)
Aerial parts included stems, leaves, and flowers	Lebanon	3 h	*β*-Caryophyllene, caryophyllene oxide, (E)-*β*-farnesene and (Z)-*β*-farnesene (41.6, 9.5, 6.2 and 4.8%, respectively)	(Mancini et al., 2009)
Aerial parts included stems, leaves, and flowers	Lebanon	3 h	*β*-Caryophyllene, caryophyllene oxide, and (E)-*β*-farnesene (50.2, 6.4, and 5.3%, respectively).	(Senatore et al., 2005)
Aerial parts included stems, leaves, and flowers	Jordan	3 h	The EO isolated from the fresh aerial parts consisted mainly of hydrocarbon sesquiterpenes (55.27%). In addition, the dried aerial parts were mainly consisting of oxygenated monoterpene (50.31%). However, the main ingredients characterized in the EOs of dried and fresh plant aerial parts were 4a*α*,7*α*,7a*α*-Nepetalactone (43.8, 17.7%, respectively), *E*-caryophyllene (11.5, 16.3%, respectively) and *γ*-murolene (10.4 and 18.54%, respectively).	(Al-Qudah, 2016)

utilized worldwide for treatment of diabetes due to their scarce side effects and traditional acceptability. Consequently, the screening of *α*-glucosidase and *α*-amylase inhibitors among herbals and other natural products has received considerable global attention (Jaradat et al., 2019). In fact, *α*-glucosidase and *α*-amylase are the major carbohydrate-hydrolyzing enzymes that are accountable for post-prandial hyperglycemia. *α*-Amylase initiates the carbohydrate digestion process by hydrolyzing 1,4-glycosidic linkage of the complex poly-saccharides (glycogen, starch) yielding disaccharide units, which in turn undergo *α*-glucosidase-catalyzed hydrolysis to monosaccharide units leading to postprandial hyperglycemia (Mrabti et al., 2018). Thera-peutically, the intake of carbohydrate digesting enzymes inhibitors plays a vital role in the control of hyperglycemia *via* reducing the intestinal glucose absorption (Spínola et al., 2020). acarbose, a leading carbohy-drate metabolic enzyme inhibitor, has several side effects such as abdominal pain, flatulence, diarrhea, bloating, and cramping (Singh et al., 2008).

Screening of the EO of *N. curviflora* extracted from leaves, and flowers revealed strong inhibition activity against porcine pancreatic *α*-amylase enzyme with an IC_{50} value of 45.7 ± 0.26 μg/mL while the anti-amylase commercial drug (acarbose) has an IC_{50} value of 28.84 ± 1.22 μg/mL. Zarrabi et al. reported that *N. racemosa* aqueous decoction displayed a strong inhibition activity against *α*-amylase and *α*-glucosidase with an IC_{50} value of 9.45, and 33.57 μmol of acarbose equivalent per gram of dried extract, respectively (Zarrabi et al., 2019).

The inhibition activity of *N. curviflora* EO on *α*-glucosidase was also studied. The EO displayed high inhibition activity against *α*-glucosidase enzyme with an IC_{50} value of 54.9 ± 0.34 μg/mL, whilst that of acarbose was 28.84 ± 1.22 μg/mL. In a study conducted by Sarikurkcua et al., EOs of *N. cadmea* and *N. nuda* subsp. *glandulifera* displayed strong *α*-amylase inhibitory activity with IC_{50} values of 1.35 ± 0.02 and 1.15 ± 0.05 mg/mL, respectively, which is very close to that of acarbose (positive control, $IC_{50} = 1.33 \pm 0.01$ mg/mL) (Sarikurkcu et al., 2018). Moreover, the same study reported that these EOs have also strong inhibition actions against *α*-glucosidase with IC_{50} values of 5.93 ± 0.18 and 0.99 ± 0.01 mg/mL, respectively, whereas that of acarbose was 0.99 ± 0.01 mg/mL (Sarikurkcu et al., 2018). The previously reported data are similar to the inhibition activity of *N. curviflora* EO against *α*-amylase and *α*-glucosidase found during the current study. To the best of our knowledge, the *α*-amylase and *α*-glucosidase inhibitory actions of *N. curviflora* EO have never been reported in the literature.

Cancer metastasis takes place when tumor cells separate from the primary tumor to spread through body tissues and blood vessels to colonize distant organs. Cell migration, one of the pivotal steps in cancer metastasis, greatly reduces the chances of survival (Xu et al., 2018). Thus, investigating cancer cell migration is an important aspect of a cancer treatment strategy. Interestingly, more than 50% of anticancer drugs were derived from natural products during the last century (Yuan et al., 2016). This study revealed that non-cytotoxic concentrations of EO have significantly reduced cancer cell migration potential of uterine cervical cancer cells within 24 h. Abdallah et al. have previously re-ported a comparable inhibitory effect on HeLa cancer cell migration following the treatment with *Rhus coriaria* plant extract in similar conditions using wound healing assay (Abdallah et al., 2019). The two studies prove a high level of responsiveness to the migratory behavior of HeLa cancer cells to the plant extracts. This study is frontier to find the effect of EO on cancer cell migration and especially on cervical cancer cells.

In the cell growth assay, it was found that increasing concentrations of EO lead to inhibition of cervical carcinoma viability after 24 h and 48 h of treatment. The concentrations of EO that proved to affect HeLa cell viability were the same between the two time points. However, the intensity of the response has increased when the treatment lasted for 48 h, especially at 0.625 mg/mL of EO where the viability was reduced by nearly 60% compared with 24 h treatment. It was observed that the IC_{50} has significantly decreased after 48 h. These findings strongly indicate that the EO effect on uterus cervix cancer cells is time-dependent. It has been previously reported that silver nanoparticles manufactured from *Nepeta deflersiana* aqueous extract, a plant from the same genus, displayed an inhibitory effect against HeLa cancer cell viability using MTT assay (Al-Sheddi et al., 2018). In the current study, the effect was observed at higher concentrations and this might be due to the plant species difference even though they belong to the same genus. The current study concludes that *N. curviflora* EO possesses inhibitory action on cervical cancer viability.

5. Conclusion

In conclusion, 1,6-dimethyl spiro[4.5]decane, caryophyllene oxide, and *β*-caryophyllene were found to be the major components of *N. curviflora* leaves EO and flowers growing in Palestinian mountains. The EO revealed potential antioxidant, antilipase, *α*-amylase, and *α*-glucosidase enzymes inhibition activities compared with the positive controls (trolox, orlistat, and acarbose, respectively). Significantly, EO proved to have an inhibitory role in cervical cancer cell migration and proliferation. These outcomes could advocate its use as a natural source

of anticancer, antioxidant, anti-obesity, and antidiabetic therapeutic agents.

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Ethical approval

N/A.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abdallah, S., Abu-Reidah, I., Mousa, A., Abdel-Latif, T., 2019. *Rhus coriaria* (sumac) extract reduces migration capacity of uterus cervix cancer cells. Rev. Bras. Farmacogn. 29, 591–596.

Al-Qudah, M.A., 2016. Antioxidant acitvity and chemical composition of essential oils of fresh and air-dried Jordanian *Nepeta curviflora* Boiss. J. Biol. Active Prod. Nat. 6, 101–111. https://doi.org/10.1080/22311866.2016.1199286.

Al-Sheddi, E.S., Farshori, N.N., Al-Oqail, M.M., Al-Massarani, S.M., Saquib, Q., Wahab, R., Musarrat, J., Al-Khedhairy, A.A., Siddiqui, M.A., 2018. Anticancer potential of green synthesized silver nanoparticles using extract of *Nepeta deflersiana* against human cervical cancer cells (HeLA). Bioinorg. Chem. Appl. 2018 https://doi.org/10.1155/2018/9390784.

Amer, J., Jaradat, N., Hattab, S., Al-hihi, S., 2019. Traditional Palestinian medicinal plant *Cercis siliquastrum* (Judas tree) inhibits the DNA cell cycle of breast cancer-antimicrobial and antioxidant characteristics. Eur. J. Integr. Med. 27, 90–96. https://doi.org/10.1016/j.eujim.2019.03.005.

Amira, S., Dade, M., Schinella, G., Ríos, J.-L., 2012. Anti-inflammatory, anti-oxidant, and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin. Pak. J. Pharm. Sci. 25, 65–72.

Barhoumi, L.M., Al-Jaber, H.I., Zarga, M.A., 2017. Volatile organic compounds and essential oil composition of selected organs of *Nepeta curviflora* collected from two regions in Jordan. Jordan J. Chem. 12, 101–112.

Bender, T., Martinou, J.C., 2013. Where killers meet-permeabilization of the outer mitochondrial membrane during apoptosis. Cold Spring Harb. Perspect. Biol. 5 https://doi.org/10.1101/cshperspect.a011106.

Buskens, E., Vogt, T.C., Liefbroer, A.C., Reijneveld, M.S., Bultmann, U., Henkens, K.C., de Ruiter, J.H., de Rooij, S.E., 2019. Healthy Ageing: Challenges and Opportunities of Demographic and Societal Transitions, Older People: Improving Health and Social Care. Springer, Germany, pp. 9–31.

Caiger, S., 2014. Essential Oils and Oleoresins. ITC's Market Insider, Geneva.

Carini, F., Tomasello, G., Jurjus, A., Geagea, A., Al Kattar, S., Damiani, P., Sinagra, E., Rappa, F., David, S., Cappello, F., 2017. Colorectal cancer and inflammatory bowel diseases: effects of diet and antioxidants. J. Biol. Regul. Homeost. Agents 31, 791–795.

Cooper, G.M., 2000. The Cell: a Molecular Approach, 2nd ed. Sinauer Associates, Sunderland.

Fidyt, K., Fiedorowicz, A., Strządała, L., Szumny, A., 2016. β-Caryophyllene and β-caryophyllene oxide-natural compounds of anticancer and analgesic properties. Cancer Med. 5, 3007–3017. https://doi.org/10.1002/cam4.816.

Guo, S., Ge, Y., Jom, K.N., 2017. A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). Chem. Cent. J. 11, 95–101. https://doi.org/10.1186/s13065-017-0328-7.

Hadi, N., Sefidkon, F., Shojaeiyan, A., Šiler, B., Jafari, A.-A., Aničić, N., Mišić, D., 2017. Phenolics' composition in four endemic *Nepeta* species from Iran cultivated under experimental field conditions: the possibility of the exploitation of *Nepeta germplasm*. Ind. Crops Prod. 95, 475–484. https://doi.org/10.1016/j.indcrop.2016.10.059.

Hawash, M., Jaradat, N., Elaraj, J., Hamdan, A., Lebdeh, S.A., Halawa, T., 2019. Evaluation of the hypoglycemic effect of seven wild folkloric edible plants from Palestine. J. Complement. Integr. Med. 2019 https://doi.org/10.1515/jcim-2019-0032.

Hecht, F., Pessoa, C.F., Gentile, L.B., Rosenthal, D., Carvalho, D.P., Fortunato, R.S., 2016. The role of oxidative stress on breast cancer development and therapy. J. Immunother. Emphasis Tumor Immunol. 37, 4281–4291. https://doi.org/10.1007/s13277-016-4873-9.

Jaradat, N.A., Abualhasan, M., 2015. Comparison in vitro of antioxidant activity between fifteen *Campanula* species (Bellflower) from Palestinian flora. Pharmacogn. J. 7 https://doi.org/10.5530/pj.2015.5535.5534.

Jaradat, N., Al-Maharik, N., 2019. Fingerprinting, antimicrobial, antioxidant, anticancer, cyclooxygenase and metabolic enzymes inhibitory characteristic evaluations of *Stachys viticina* Boiss. Essential oil. Molecules 24. https://doi.org/10.3390/molecules24213880.

Jaradat, N., Adwan, L., K'aibni, S., Shraim, N., Zaid, A.N., 2016a. Chemical composition, anthelmintic, antibacterial and antioxidant effects of *Thymus bovei* essential oil. BMC Complement. Altern. Med. 16 https://doi.org/10.1186/s12906-12016-11408-12902.

Jaradat, N.A., Zaid, A.N., Hussein, F., 2016b. Investigation of the antiobesity and antioxidant properties of wild *Plumbago europaea* and *Plumbago auriculata* from North Palestine. Chem. Biol. Technol. Agric. 3, 1–9. https://doi.org/10.1186/s40538-40016-40082-40534.

Jaradat, N., Zaid, A., Hussein, F., Zaqzouq, M., Aljammal, H., Ayesh, O., 2017. Anti-lipase potential of the organic and aqueous extracts of ten traditional edible and medicinal plants in Palestine; a comparison study with orlistat. Medicines 4. https://doi.org/10.3390/medicines4040089.

Jaradat, N.A., Al-lahham, S., Zaid, A.N., Hussein, F., Issa, L., Abualhasan, M.N., Hawash, M., Yahya, A., Shehadi, O., Omair, R., 2019. *Carlina curetum* plant phytoconstituents, enzymes inhibitory and cytotoxic activity on cervical epithelial carcinoma and colon cancer cell lines. Eur. J. Integr. Med. 30 https://doi.org/10.1016/j.eujim.2019.100933.

Kaska, A., Deniz, N., Çiçek, M., Mammadov, R., 2018. Evaluation of antioxidant properties, phenolic compounds, anthelmintic, and cytotoxic activities of various extracts isolated from *Nepeta cadmea*: an endemic plant for Turkey. J. Food Sci. 83 https://doi.org/10.1111/1750-3841.14167.

Kushner, R.F., 2019. Obesity and Therapeutic Approaches to Weight Loss, Comprehensive Cardiovascular Medicine in the Primary Care Setting. Springer, Germany, pp. 71–85.

Kwon, C.-S., Sohn, H.Y., Kim, S.H., Kim, J.H., Son, K.H., Lee, J.S., Lim, J.K., Kim, J.-S., 2003. Anti-obesity effect of *Dioscorea nipponica* Makino with lipase-inhibitory activity in rodents. Biosci. Biotech. Bioch. 67, 1451–1456. https://doi.org/10.1271/bbb.67.1451.

Malik, T., 2018. Perspective uses of essential oils in functional foods and antimicrobial packaging material. Food Sci. Nutr. 17, 231–235. https://doi.org/10.4018/978-15225-0607-2.ch010.

Mancini, E., Apostolides Arnold, N., De Feo, V., Formisano, C., Rigano, D., Piozzi, F., Senatore, F., 2009. Phytotoxic effects of essential oils of *Nepeta curviflora* Boiss. and *Nepeta nuda*L. subsp. albiflora growing wild in Lebanon. J. Plant Interact. 4, 253–259. https://doi.org/10.1080/17429140903225507.

Mitra, A., Dewanjee, D., Dey, B., 2012. Mechanistic studies of lifestyle interventions in type 2 diabetes. World J. Diabetes 3. https://doi.org/10.4239/wjd.v4233.i4212.4201.

Mrabti, H.N., Sayah, K., Jaradat, N., Kichou, F., Ed-Dra, A., Belarj, B., Cherrah, Y., Faouzi, M.E.A., 2018. Antidiabetic and protective effects of the aqueous extract of *Arbutus unedo* L. In streptozotocin-nicotinamide-induced diabetic mice. J. Complement. Integr. Med. 15 https://doi.org/10.1515/jcim-2017-0165.

Musso, L., Scaglia, B., Haj, G.A., Arnold, N.A., Adani, F., Scarì, G., Dallavalle, S., Iriti, M., 2017. Chemical characterization and nematicidal activity of the essential oil of*Nepeta nuda* L. ssp. pubescens and *Nepeta curviflora* Boiss. from Lebanon. J. Essent. Oil Bear. Pl. 20, 1424–1433. https://doi.org/10.1080/0972060X.2017.1407678.

Ndhlala, A.R., Moyo, M., Van Staden, J., 2010. Natural antioxidants: fascinating or mythical biomolecules? Molecules 15, 6905–6930. https://doi.org/10.3390/molecules15106905.

Olokoba, A.B., Obateru, O.A., Olokoba, L.B., 2012. Type 2 diabetes mellitus: a review of current trends. Oman Med. J. 27, 269–274. https://doi.org/10.5001/omj.2012.68.

Patterson, R.E., White, E., Kristal, A.R., Neuhouser, M.L., Potter, J.D., 1997. Vitamin supplements and cancer risk: the epidemiologic evidence. Cancer Causes Control 8, 786–802. https://doi.org/10.1023/a:1018443724293.

Poprac, P., Jomova, K., Simunkova, M., Kollar, V., Rhodes, C.J., Valko, M., 2017. Targeting free radicals in oxidative stress-related human diseases. Trends Pharmacol. Sci. 38, 592–607. https://doi.org/10.1016/j.tips.2017.04.005.

Reis, J.F., Monteiro, V.V.S., Gomes, R.S., Carmo, M.M., Costa, G.V., Ribera, P.C., Monteiro, M.C., 2016. Action mechanism and cardiovascular effect of anthocyanins: a systematic review of animal and human studies. J. Transl. Med. 14, 315–322. https://doi.org/10.1186/s12967-016-1076-5.

Roh, C., Jung, U., 2012. *Nepeta japonica* Maximowicz extract from natural products inhibits lipid accumulation. J. Sci. Food Agric. 92, 2195–2199. https://doi.org/10.1002/jsfa.5608.

Salameh, N., Shraim, N., Jaradat, N., 2018. Chemical composition and enzymatic screening of *Micromeria fruticosa serpyllifolia* volatile oils collected from three different regions of West Bank. Palestine. Biomed. Res. Int. 2018 https://doi.org/10.1155/2018/6536919.

Santini, A., Tenore, G.C., Novellino, E., 2017. Nutraceuticals: a paradigm of proactive medicine. Eur. J. Pharm. Sci. 96, 53–61. https://doi.org/10.1016/j.ejps.2016.09.003.

Sarikurkcu, C., Ceylan, O., Targan, S., Zeljković, S.Ć., 2018. Chemical composition and biological activities of the essential oils of two endemic *Nepeta* species. Ind. Crops Prod. 125, 5–8. https://doi.org/10.1016/j.indcrop.2018.1009.1001.

Seiler, A., Chen, M.A., Brown, R.L., Fagundes, C.P., 2018. Obesity, dietary factors, nutrition, and breast cancer risk. Curr. Breast Cancer Rep. 10, 1–14. https://doi.org/10.1007/s12609-018-0264-0.

Senatore, F., Arnold, N.A., Piozzi, F., 2005. Composition of the essential oil of *Nepeta curviflora* Boiss.(Lamiaceae) from Lebanon. J. Essent. Oil Res. 17, 268–270. https://doi.org/10.1080/10412905.2005.9698898.

Serfling, A., Wohlrab, J., Deising, H.B., 2007. Treatment of a clinically relevant plant-pathogenic fungus with an agricultural azole causes cross-resistance to medical azoles and potentiates caspofungin efficacy. Antimicrob. Agents Chemother. 51, 3672–3676. https://doi.org/10.1128/AAC.00654-07.

Shafaghat, A., Oji, K., 2010. Nepetalactone content and antibacterial activity of the essential oils from different parts of *Nepeta persica*. Nat. Prod. Commun. 5, 625–628.

Shahri, S.M.M., Jafari, A., Mahmoodzadeh, H., 2016. Comparative anatomical studies on petioles of *Nepeta* L. Species (Lamiaceae) in NE Iran. Adv. Stud. Biol. 8, 119–126.

Sharifi-Rad, J., Sureda, A., Tenore, G.C., Daglia, M., Sharifi-Rad, M., Valussi, M., Tundis, R., Sharifi-Rad, M., Loizzo, M.R., Ademiluyi, A.O., 2017. Biological activities of essential oils: from plant chemoecology to traditional healing systems. Molecules 22, 70–81. https://doi.org/10.3390/molecules22010070.

Sikka, S.C., Bartolome, A.R., 2018. Perfumery, essential oils, and household chemicals affecting reproductive and sexual health. Bioenvironmental Issues Affecting Men's Reproductive and Sexual Health. Elsevier, Netherlands, pp. 557–569.

Singh, S.K., Rai, P.K., Jaiswal, D., Watal, G., 2008. Evidence-based critical evaluation of glycemic potential of *Cynodon dactylon*. Evid. Complement. Alternat. Med. 5, 415–420. https://doi.org/10.1093/ecam/nem044.

Skorić, M., Gligorijević, N., Čavić, M., Todorović, S., Janković, R., Ristić, M., Mišić, D., Radulović, S., 2017. Cytotoxic activity of *Nepeta rtanjensis* Diklić & Milojević essential oil and its mode of action. Ind. Crops Prod. 100, 163–170 https://doi.org/110.1016/j.indcrop.2017.1002.1027.

Spínola, V., Llorent-Martínez, E.J., Castilho, P.C., 2020. Inhibition of α-amylase, α-glucosidase and Pancreatic Lipase by Phenolic Compounds of *Rumex maderensis* (Madeira Sorrel). Influence of Simulated Gastrointestinal Digestion on Hyperglycaemia-related Damage Linked With Aldose Reductase Activity and Protein Glycation. LWT 118. https://doi.org/10.1016/j.lwt.2019.108727.

Tas, S., Tas, B., Bassalat, N., Jaradat, N., 2018. In-vivo, hypoglycemic, hypolipidemic and oxidative stress inhibitory activities of *Myrtus communis* L. Fruits hydroalcoholic extract in normoglycemic and streptozotocin-induced diabetic rats. Biomed. Res. 29 https://doi.org/10.4066/biomedicalresearch.4029-4018-4708.

Vinaixa, M., Schymanski, E.L., Neumann, S., Navarro, M., Salek, R.M., Yanes, O., 2016. Mass spectral databases for LC/MS-and GC/MS-based metabolomics: state of the field and future prospects. TrAC Trend. Anal. Chem. 78, 23–35. https://doi.org/10.1016/j.trac.2015.1009.1005.

Wei, X., Koo, I., Kim, S., Zhang, X., 2014. Compound identification in GC-MS by simultaneously evaluating the mass spectrum and retention index. Analyst 139, 2507–2514. https://doi.org/10.1039/c3an02171h.

Xu, L., Gordon, R., Farmer, R., Pattanayak, A., Binkowski, A., Huang, X., Avram, M., Krishna, S., Voll, E., Pavese, J., 2018. Precision therapeutic targeting of human cancer cell motility. Nat. Commun. 9, 1–14. https://doi.org/10.1038/s41467-41018-04465-41465.

Yuan, H., Ma, Q., Ye, L., Piao, G., 2016. The traditional medicine and modern medicine from natural products. Molecules 21. https://doi.org/10.3390/molecules21050559.

Zarrabi, M., Asghari, B., Maryamabadi, A., Mohebbi, G., Rashvand, S., 2019. Phytochemical properties and inhibitory and antioxidant effects of the decoction, infusion and hydro-alcoholic extract of *Nepeta racemosa* on α-amylase and α-glucosidase. Iran South Med. J. 22, 90–105.