Arabian Journal for Science and Engineering
https://doi.org/10.1007/s13369-019-03980-x

RESEARCH ARTICLE - BIOLOGICAL SCIENCES

Chemical Fingerprinting, Anticancer, Anti-inflammatory and Free Radical Scavenging Properties of *Calamintha fenzlii* Vis. Volatile Oil from Palestine

Nidal Jaradat[1] · Saad Al-lahham[2] · Murad N. Abualhasan[1] · Duaa Ghannam[1] · Khansa Mousa[1] · Hala Kolayb[1] · Fatima Hussein[1] · Linda Issa[1] · Ahmed Mousa[2]

Received: 29 December 2018 / Accepted: 19 June 2019

Abstract
Many phytochemicals have medicinal properties including anti-inflammatory, antioxidant and cytotoxic activities. The current study aims to estimate and identify the chemical composition of *Calamintha fenzlii* Vis. leaves volatile oil and to evaluate its cytotoxic, anti-inflammatory and antioxidant properties. The volatile oil (VO) of *C. fenzlii* leaves was separated using microwave ultrasonic method and the chemical composition was characterized using Gas Chromatography–Mass Spectroscopy method. The anti-inflammatory, antioxidant and cytotoxic activities were determined using a COX inhibitor screening, the DPPH-free radical scavenging and MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-cyrboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) cell viability colorimetric assays, respectively. The chemical constituents of *C. fenzlii* VO were dominated by oxygenated monoterpenoid (96.91%). The major chemical components of *C. fenzlii* VO were represented by menthone 68.93% and pulegone 23.1%. The cytotoxicity test results revealed that the 23.22, 11.6, 5.8 and 2.9 mg/ml of *C. fenzlii* VO treatments induced cell death significantly ($p \leq 0.0001$) by 70%, while 1.4 and 0.7 mg/ml induced cell cytotoxicity significantly ($p \leq 0.0001$) by approximately 50% and 40%, respectively. The VO showed moderate antioxidant activity with an IC_{50} value of 15.38 ± 0.81 μg/ml. The COX-1 IC_{50} value was 0.215 μg/ml, while COX-2 IC_{50} was 0.241 μg/ml. Our data demonstrate for the first time that the VO of the Palestinian *C. fenzlii* plant possesses cytotoxic, anti-inflammatory and antioxidants effects. It is a good source of therapeutic active compounds that could have potential applications in the nutraceutical and pharmaceutical industries.

Keywords *Calamintha fenzlii* · Antioxidant · Cyclooxygenase · Cytotoxicity · Volatile oil

1 Introduction

From ancient times, plants and their derivatives were used as medicine and cosmetics. In 5000 BC, Sumerian used thyme in medicine, while in 1550 BC, Egyptians documented through the papyri material the use of fennel, juniper, cumin and garlic to keep their healthy lifestyle [1]. Nowadays, more than 70% of the populations in underdeveloped and developing countries have faith in traditional medicine and herbal remedies [2]. For that, this increased attention has been paid pharmaceutical companies to produce in huge quantities herbal supplements and natural therapeutic agents. In view of the fact that these products are more economical, friendly to the environment and have fewer side effects caused by synthetic chemicals [3].

Volatile oils (VOs) are one of the common forms of natural products which were isolated from different plants parts, some animals and fungi. In traditional medicine, these oils were used for various therapeutic applications such as bactericidal, fungicidal, parasiticidal, antitussive and counter-irritants. Nowadays, in addition to their therapeutic applications, they were used widely in cosmetics, sanitary, agriculture and food industries [4]. Volatile oils are widely used in food preservation and disease prevention, as they possess antioxidant property. They detoxify reactive oxygen

✉ Nidal Jaradat
nidaljaradat@najah.edu

[1] Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, P.O. Box 7, Nablus, Palestine

[2] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, P.O. Box 7, Nablus, Palestine

Published online: 28 June 2019

species that rise under certain pathological conditions leading to oxidative stress, which is the main risk factor for cancer, type-2 diabetes, cardiovascular and neurological diseases [5]. Furthermore, VO, as a natural antioxidant, will be the alternative to the synthetic antioxidant, like BHA and BHT, that have a carcinogenic effect [6, 7].

In addition to the ability of volatile oils in scavenging of the free harmful radicals, it also has an anti-inflammatory effect by several mechanisms of action including the production of prostaglandins. Prostaglandins are acidic lipids produced from cell membranes. The primary and the rate-limiting enzyme responsible for prostaglandin synthesis is cyclooxygenase (COX) which has two forms, COX-1 and COX-2. The constitutively expressed COX-1 is present in cells under physiological conditions and maintains mucosal blood flow, promotes mucous secretion, inhibits neutrophil adherence and maintains renal blood flow. In contrast to COX-1, COX-2 is effectively absent in healthy tissue and is induced in migratory and other cells by proinflammatory agents, such as cytokines, mitogens and endotoxins under pathological conditions such as inflammation [8]. Therefore, looking for selective inhibitors against COX-2 will avoid the undesirable gastrointestinal side effects of NSAIDs (non-steroidal anti-inflammatory drugs) which non-selectively suppress COX enzymes. For that, there is greater interest in the VOs to be alternative for NSAIDs, that considered to be effective, safer and suitable for chronic and long-term use without side effects which produced by NSAIDs [9, 10].

Calamintha fenzlii Vis. (Lamiaceae family) commonly known as squaw mint, pudding grass and mosquito plant. It is a perennial herbaceous and flowering plant species which native to the Eastern parts of the Middle East, northern regions of Africa, central and southern countries of Europe and Eastern regions of Asia. It can reach height up to 15 cm, with indefinite width due to fast growing and spreading nature. The plant leaves have a dark green color and oval shape, with serrated margins and small hair on both sides. While the flowers are mauve, tiny and present late in spring and grow up the square stem and extend out from near the node of the leaf [11].

In traditional medicine, *C. fenzlii* utilized for indigestion, coughs, fevers, kidney and liver problems and also used for the treatment of headaches, tuberculosis, influenza and smallpox [12–16].

To the best of author's knowledge, there have been no previous studies conducted on the antioxidant, anti-inflammatory, cytotoxic effect and identification of the chemical constituents of the VO extracted from *C. fenzlii* leaves from Palestine by ultrasonic microwave cooperative extractor apparatus and the current study is the first one which deals with these objectives.

2 Materials and Methods

2.1 Equipment

The ultrasonic microwave cooperative extractor/reactor (Lab-Kits, UM2015042801A, CW-2000, Hong Kong, China), spectrophotometer (Jenway, UV/Vis Spectrophotometer 6505, UK), micropipettes (Finnpipette, Finland), CO_2 incubator (ESCO, 2012-74317, Singapore), 96-well plates (Greiner bio-one, North America), horizontal laminar flow (MRC, BBS12HGs, Israel), shaking laboratory water bath (Lab Tech, BPXOP1001040, Namyangju, South Korea), microplate reader (Unilab, RTC 6000, USA), inverted biological microscope (MRC, XDS-2, Wuzhou, China), electronic balance (Wagl, AS 220/C/2, Radwag, Poland), ELISA plate reader (BioRad, 680 XR, Japan), COX inhibitor screening assay kit (Cayman Chemical, 560131, USA) were used in this study.

2.2 Chemicals

Methanol (Loba Chemie, India), Trolox ((s)-(–)-6 hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) (Sigma-Aldrich, 391921-1G, Denmark), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, D9132-1G, Germany), Dimethyl sulfoxide (DMSO) (Riedel-de-haen, Q4199, Germany), Trypsin–EDTA solution 1x (Sigma-Aldrich, 59417C, USA), RPMI-1640 medium (Roswell Park Memorial Institute-1640 medium) (Sigma-Aldrich, R0883, UK), MTS (Manothermosonication) (Spanish, 9200686, Spain), PBS (phosphate buffer saline) (Sigma-Aldrich, 79383, Germany). CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay (Promega Corporation, Madison, USA) and Elisa kit (Brookhaven, 280173, New York, USA) were used in this study.

2.3 *C. fenzlii* Leaves Collection and Preparation

The leaves of *C. fenzlii* were collected in April 2017 from the Nablus region of Palestine. A small sample of *C. fenzlii* plant was identified by the pharmacognosist Dr. Nidal Jaradat and the herbarium specimen was kept in the Pharmacognosy and Herbal products laboratory at An-Najah National University under the voucher specimen code number: Pharm-PCT-1567.

The leaves were dried in the shade at room temperature for 2 weeks until all the plant leaves become well dried. After drying, the plant materials were powdered using a mechanical grinder and then transferred into an airtight container with proper labeling for future use.

2.4 Preparing the VO

For *C. fenzlii* VO extraction, a microwave ultrasonic apparatus was utilized by placing 100 g of the dried plant leaves in a special round bottom flask with 250 ml of distilled water in the apparatus, the microwave oven was adjusted at 1000 W power while the ultrasonic power was adjusted at 50 W and at a frequency of 40 kHz. This isolation procedure was carried out for 30 min at 100 °C. This procedure was repeated three times. The obtained VO was collected into a clean beaker, chemically dried with $CaCO_3$ and then kept in dark amber colored bottle in the refrigerator at − 5 °C. The VO yield was 0.87% w/w based on the dried weight.

2.5 Antioxidant Activity

The stable DPPH molecule (2,2-diphenyl-1-picrylhydrazyl) was utilized for the estimation of the antioxidant activity of *C. fenzlii* VO. One ml of VO with different concentrations diluted in methanol was added to 1 ml of DPPH methanolic solution (0.002% w/v). After a 30 min of incubation at room ordinary temperature, the absorbance was read against a blank at 517 nm by utilizing UV–Vis spectrophotometer. The percentage (%) to inhibit DPPH· free radical was determined using the following formula:

$$\text{DPPH} \cdot \text{inhibition potential}(\%) = [(C - T)/C] \times 100.$$

where C is the absorbance of the control (without VO), T is the absorbance of the tested VO, Trolox was used as posi-

Table 1 The chemical constituents of *C. fenzlii* VO

Volatile oil	RT	RI	Area	%	Classes
Menthone	6.38	976	4687892	68.93	Oxygenated monoterpenoid
Pulegone	8.84	869	1571044	23.1	Oxygenated monoterpenoid
Isomenthone	6.64	786	202284	2.97	Oxygenated monoterpenoid
Caryophllene	15.38	844	183854	2.7	Hydrocarbon sesquiterpenoid
Piperitone	9.36	879	129758	1.91	Oxygenated monoterpenoid
Germacrene D	17.69	671	26278	0.39	Hydrocarbon sesquiterpenoid
SUM			6801110	100	

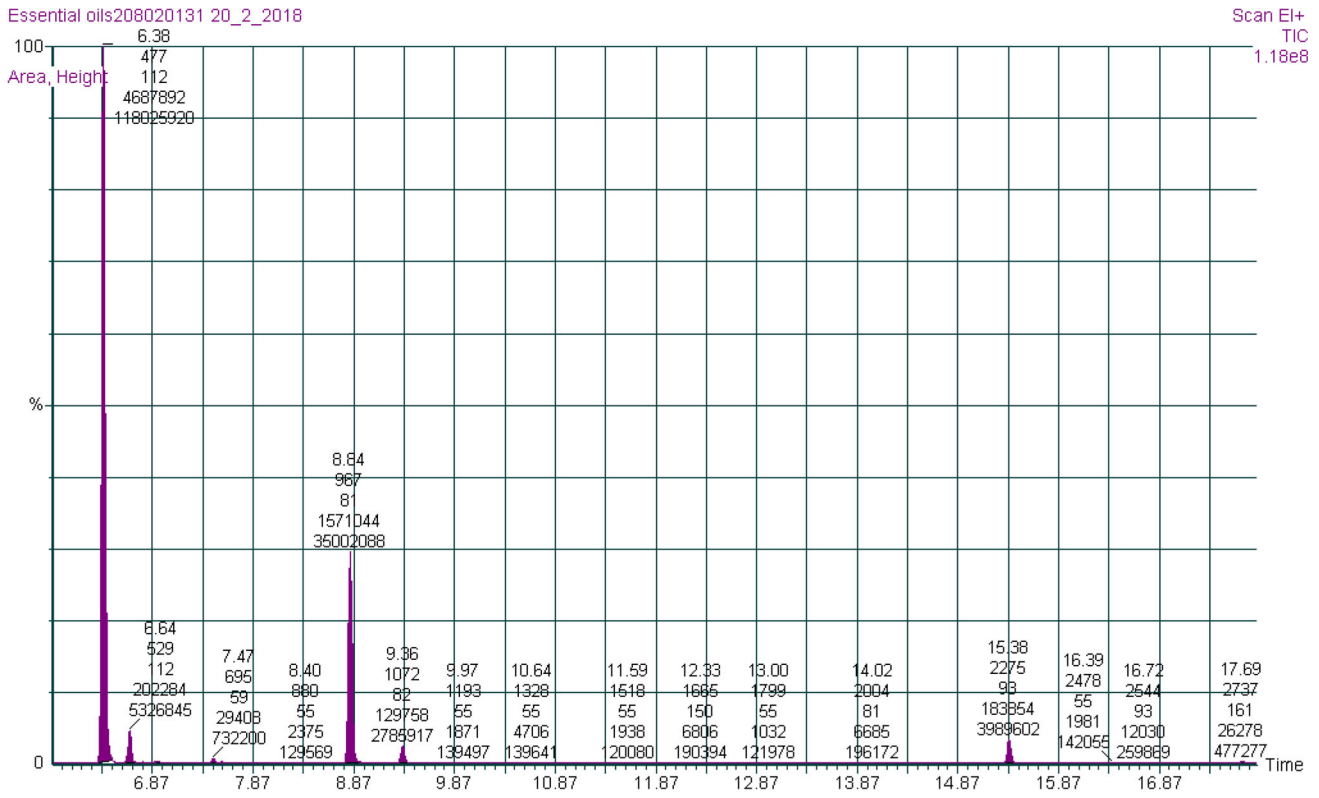

Fig. 1 GC-MS chromatogram of *C. fenzlii* VO

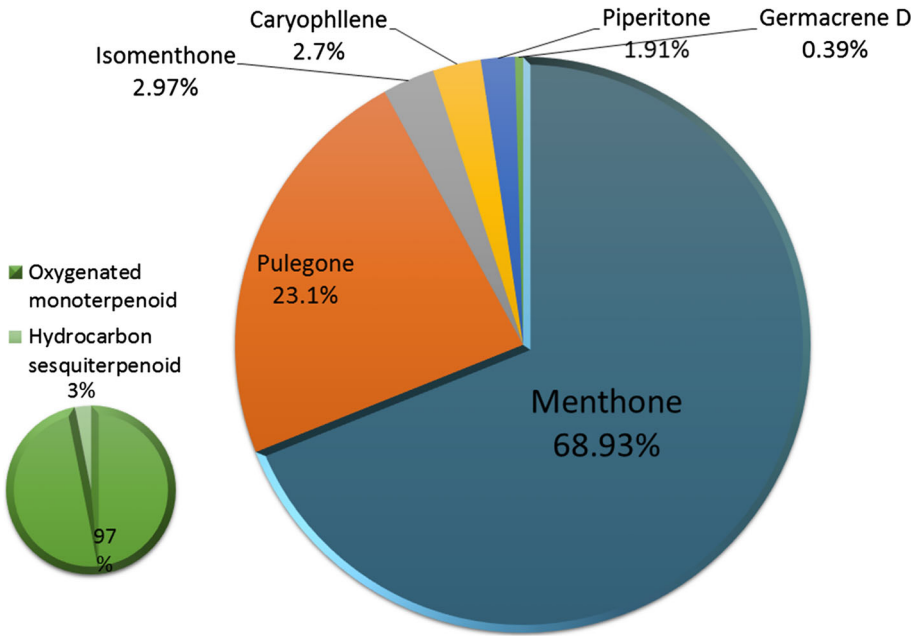

Fig. 2 The VO composition of *C. fenzlii*

tive control and VO concentrations providing 50% inhibition (IC_{50}) were estimated by plotting the percentages of inhibition against the concentrations of the sample. All the tests were repeated in triplicate, and the IC_{50} values were stated as mean ± SD [17].

2.6 Determination of COX-2 Inhibition

The ability of the *C. fenzlii* VO to prevent the conversion of Arachidonic Acid (AA) to PGH_2 by human recombinant COX-2 and bovine COX-1 was assessed using a COX inhibitor screening assay kit (Item No: 560131) according to the Cayman chemical manufacturer guidelines (USA). The inhibitory concentration 50 (IC_{50}) of COX-1/COX-2 activity of *C. fenzlii* VO was done in which the assay was run, in duplicate, with two concentrations of the crude plant (0.01 and 1 µg/ml). A standard curve of eight concentrations, a non-specific binding sample, and a maximum binding sample were used as instructed in the kit manual to determine the inhibition of sample plant applying the generated multiple regression best-fit line. The percentage inhibition of the two concentrations was used to calculate the IC_{50}.

2.7 Cytotoxicity Assay

HeLa cervical adenocarcinoma cells were cultured in RPMI-1640 media supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin antibiotics and 1% L-glutamine. Cells were grown in a humidified atmosphere with 5% CO_2 at 37 °C. Cells were seeded at 2.6×10^4 cells/well in a 96-well plate. After 48 h, cells were confluent, media were changed and cells were incubated with or without 23.22, 11.6, 5.8, 2.9, 1.4, 0.7, 0.38 and 0.18 mg/ml of *C. fenzlii* VO. Cell viability was assessed by CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the Promega Corporation manufacturer instructions (USA).

2.8 Statistical Analyses

Determination of the antioxidant activity was carried out in triplicate for each sample. The obtained results were presented as mean ± standard deviation (SD).

3 Results

3.1 Chemical Composition

The chemical constituents of the Palestinian *C. fenzlii* plant VO were identified and estimated by the GC–MS method. Six molecules have been identified representing 100% of total VO. The chemical constituents of *C. fenzlii* VO were dominated by oxygenated monoterpenoid (96.91%), which included menthone 68.93%, pulegone 23.1%, isomenthone 2.97% and piperitone 1.91%. The remaining constituents were hydrocarbon sesquiterpenoid, which included caryophyllene 2.7% and germacrene D 0.39%. The *C. fenzlii* VO components and the respective percentage content are shown in Table 1 and presented in Figs. 1 and 2.

3.2 Antioxidant Potential

The antioxidant activity of the Palestinian *C. fenzlii* VO was estimated using in vitro antioxidant DPPH· assay and com-

Table 2 The IC_{50} values and percentage inhibition of DPPH activity by *C. fenzlii* VO and Trolox

Concentrations	Trolox	±SD	*C. fenzlii* VO	±SD
0	0	0	0	0
1	38.6	1.23	12.65	1.03
2	49.5	1.75	15.9	0.55
3	59.8	0.23	21	1.7
5	78.8	1.62	27.66	2.2
7	88	2.1	33.7	0.88
10	97.2	1.52	39.8	1.12
20	97.3	1.85	42.15	0
30	97.5	1.3	69.81	0.76
40	97.6	0.82	75.45	0
50	97.2	1.34	75.9	0.75
80	97.2	1.25	75.9	0.41
100	97.2	1.4	77.11	0.33
IC_{50} (µg/ml)	2.08	0.07	15.38	0.81

Table 3 *C. fenzlii* VO COX inhibition activity

Conc., µg/ml	%COX-2 inhibition, SD	%COX-1 inhibition, SD
0.25	57.8 ± 0.51	77 ± 0.82
0.5	98.1 ± 0.91	99 ± 0.97

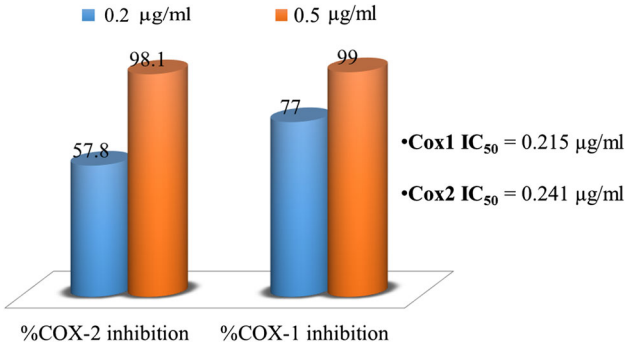

Fig. 4 Anti-inflammatory effect of *C. fenzlii* VO

pared with the synthetic reference antioxidant compound (Trolox). The results of DPPH inhibition activity of *C. fenzlii* VO are shown in Table 2 and Fig. 3. The scavenging property of all of the studied VO various concentrations samples and Trolox standard compound showed a concentration dependence property in most cases. IC_{50} of *C. fenzlii* VO and Trolox were 15.38 and 2.08 µg/ml, respectively.

3.3 Cyclooxygenase Inhibitory Assessment

The enzyme inhibition activity of the *C. fenzlii* VO was carried out using ELISA kit. The calculated percentage inhibition for the tested compounds is shown in Table 3. The calculated IC_{50} for COX-1 was found to be 0.215 µg/ml, while the IC_{50} for COX-2 was found to be 0.241 µg/ml as presented in Fig. 4.

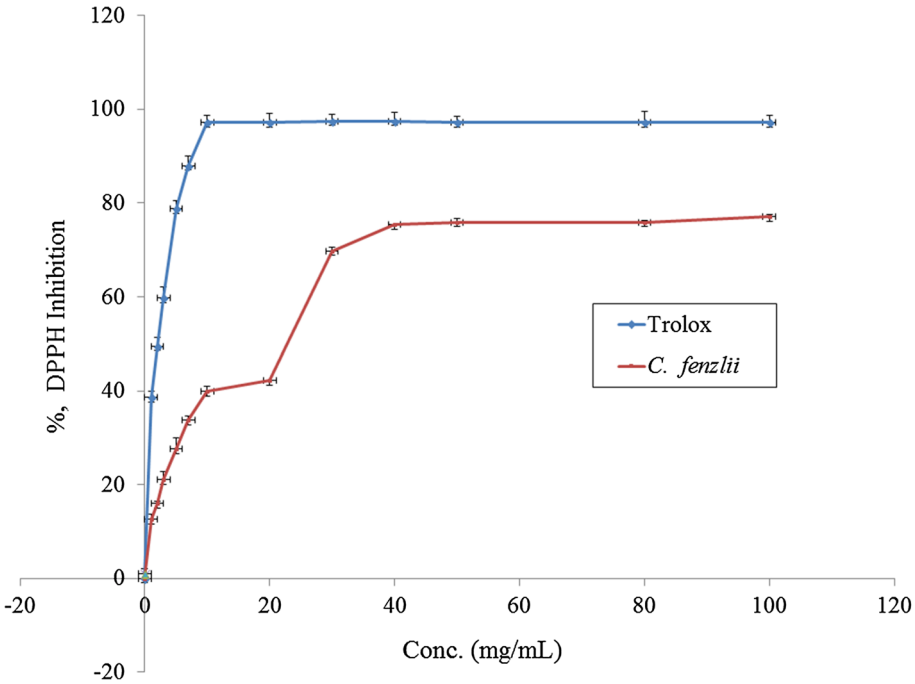

Fig. 3 The antioxidant activity of Trolox standard and *C. fenzlii* VO

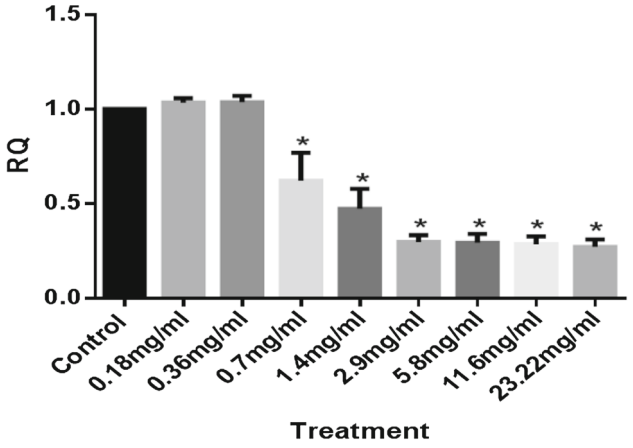

Fig. 5 *C. fenzlii* VO effect on cell cytotoxicity. Cytotoxicity was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (with only media; C) (error bars represent mean ± SD, $*p < 0.0001$)

3.4 Cytotoxicity Assay

MTS assay was used to determine the viability of HeLa cervical adenocarcinoma cells. As shown in Fig. 5, a 23.22, 11.6, 5.8 and 2.9 mg/ml of *C. fenzlii* VO treatment induced cell cytotoxicity significantly ($p \leq 0.0001$) by approximately 70%, while 1.4 and 0.7 mg/ml of *C. fenzlii* VO treatment induced cell cytotoxicity significantly ($p \leq 0.0001$) by approximately 50% and 40%, respectively. However, 0.36 and 0.18 mg/ml had no effect. HeLa cells were treated in triplicate with various concentrations of *C. fenzlii* VO, 23.22, 11.6, 5.8, 2.9 1.4 and 0.7 mg/ml, for 24 h. This experiment was repeated five times using two different *C. fenzlii* VO collections and preparations. *C. fenzlii* VO suppressed the mitochondrial activity of HeLa cells. The mechanism of this assay is based on the reduction of MTS tetrazolium compound into a colored formazan product. This conversion is accomplished by NADPH or NADH produced by mitochondrial dehydrogenase enzymes in metabolically active and living cells. Therefore, the color intensity of the formazan dye is correlated to the number of metabolically active viable cells.

4 Discussion

The VOs of Lamiaceae family species are utilized widely in cosmetics, perfumes and pharmaceutical industries. Also used as flavoring agents, preservatives in several types of foods and utilized as traditional therapeutic agents. One of these species is *C. fenzlii*, which is rich in VO and used as a traditional medicine in Palestine [18, 19].

In our study, we utilized microwave ultrasonic method for the isolation of the VO from *C. fenzlii* leaves. It is an efficient method due to the short time of extraction and no need for organic solvent, which will reduce changes and/or hydrolysis in the chemical composition of the VO [20]. The chemical composition of the current used *C. fenzlii* VO was dominated by oxygenated monoterpenoid (96.91%), which is composed of menthone 68.93%, pulegone 23.1% and isomenthone 2.97%. The constituents of the *C. fenzlii* VO growing in Palestine appeared to be different from others growing elsewhere. For example, the Portuguese *C. fenzlii* VO major constituents were pulegone 90.3% and menthone 3.6% [21], while Moroccan *C. fenzlii* VO main constituents were pulegone (40.98%) and the menthone (21.164%) [22]. This difference can be explained by a technical difference (e.g., isolation method of VO) and/or according to the types of soils, seasons and climate. In fact, these factors also affect the antioxidant activity as well as other biological activities of the isolated VO.

Indeed, the VO of *C. fenzlii* from Palestine has a stronger antioxidant activity than the ones from other countries. The Palestinian IC_{50} value was 15.38 ± 0.81 µg/ml, while Moroccan, Algerian and Iranian IC_{50} values were 58.27, 69.60 and 84.03 µg/ml, respectively [22–24]. This suggests that the Palestinian was the strongest.

In fact, cancer, oxidative stress and inflammatory diseases have become prominent in morbidity and mortality rates and still increasing at an alarming rate. Naturally derived medications have fewer side effects and more acceptable for patients in comparison with synthetic drugs [25].

The IC_{50} values for COX-1 and COX-2 are 0.215 and 0.241 µg/ml, respectively. The results demonstrate that COX enzyme inhibition of the *C. fenzlii* VO has a better inhibition activity for both COX-1 and COX-2 compared to commonly used NSAID. The IC_{50} for COX-1 of the tested plant was approximately 10 fold more than ibuprofen and aspirin (1 and 5 µg/ml), respectively. The plant VO has 100 more inhibition activity toward COX-2 compared to Aspirin and Ibuprofen (210 and 46 µg/ml), respectively. The *C. fenzlii* VO demonstrates selectivity for COX-2 enzyme; the COX-1/COX-2 ratio of the current study result was approximately 0.89 which is less than 1 and this suggests that our plant is more selective against COX-2 than COX1.

Several studies have shown the effect of Lamiaceae plants extracts on cell growth, and it has been shown that extracts derived from *C. fenzlii* exhibited low cytotoxicity property [26]. However, the studies regarding VO derived from *C. fenzlii* are scarce. VO derived from various Calamintha species, but not from *C. fenzlii,* showed an inhibitory effect on cell viability ranged from 91 to 97% at 0.5 mg/mL. Shirazi et al. [27] showed a strong cytotoxic effect of VO derived from *C. fenzlii* on various cell lines (IC_{50} 10–59 µg/ml) and is consequently considered as potentially toxic agents [28]. On the contrary, we found a weaker cytotoxic effect (at ≥ 0.7 mg/ml). This discrepancy could be due to different experimental setup or

may be due to various environmental factors, such as climate and/or seasonal factors.

However, further pharmacological, clinical and pharmaceutical investigations required to approve these biological effects in vivo and to design suitable dosage form from this natural product.

5 Conclusion

Our findings showed that the VO of the Palestinian *C. fenzlii* has anti-inflammatory, antioxidant and cytotoxic effects; however, to a higher extent than other *C. fenzlii* originates from other countries. This is most probably due to the different amount of its chemical components. The weak observed cytotoxic effect suggests that our plant is safe to use and its anticancer effect is most probably protective by inhibition of oxidative stress and inflammation. Nevertheless, the obtained data of the current study may serve as a guideline for the isolation, identification, validation and standardization of natural medications containing *C. fenzlii* VO as a main therapeutic ingredient.

Acknowledgements The authors wish to thank An-Najah National University for its support to carry out this work.

References

1. Dubey, N.; Kumar, R.; Tripathi, P.: Global promotion of herbal medicine: India's opportunity. Curr. Sci. **86**, 37–41 (2004)
2. Panday, D.R.; Rauniar, G.P.: Effect of root-extracts of *Ficus benghalensis* (Banyan) in pain in animal models. Neurosci. Rural Pract. **7**(2), 210–2017 (2016)
3. Pan, S.-Y.; Zhou, S.-F.; Gao, S.-H.; Yu, Z.-L.; Zhang, S.-F.; Tang, M.-K.; Sun, J.-N.; Ma, D.-L.; Han, Y.-F.; Fong, W.-F.: New perspectives on how to discover drugs from herbal medicines: CAM's outstanding contribution to modern therapeutics. Evid. Based Complement Altern. Med. **2013**, 22–28 (2013)
4. Pavela, R.; Benelli, G.: Essential oils as ecofriendly biopesticides? Challenges and constraints. Trends Plant Sci. **21**(12), 1000–1007 (2016)
5. Griffiths, K.; Aggarwal, B.B.; Singh, R.B.; Buttar, H.S.; Wilson, D.; De Meester, F.: Food antioxidants and their anti-inflammatory properties: a potential role in cardiovascular diseases and cancer prevention. Diseases **4**(3), 28 (2016)
6. Taghvaei, M.; Jafari, S.M.: Application and stability of natural antioxidants in edible oils in order to substitute synthetic additives. J. Food Sci. Technol. **52**(3), 1272–1282 (2015)
7. Jaradat, N.A.; Abualhasan, M.; Ali, I.: Comparison of anti-oxidant activities and exhaustive extraction yields between wild and cultivated *Cyclamen persicum, Malva sylvestris* and *Urtica pilulifera* leaves. Appl. Pharm. Sci. **5**(04), 101–106 (2015)
8. Taylor, J.; Van Staden, J.; Jäger, A.: COX-1 and COX-2 inhibitory activity in extracts prepared from Eucomis species, with further reference to extracts from *E. autumnalis* autumnalis. S. Afr. J. Bot. **68**(1), 80–85 (2002)
9. Gagnier, J.J.; Van Tulder, M.; Berman, B.; Bombardier, C.: Herbal medicine for low back pain. Cochrane Database Syst. Rev. **23**(12), 1–12 (2006)
10. Saha, S.K.; Lee, S.B.; Won, J.; Choi, H.Y.; Kim, K.; Yang, G.-M.; Dayem, A.A.; Cho, S.-G.: Correlation between oxidative stress, nutrition, and cancer initiation. Int. J. Mol. Sci. **18**(7), 1544–1549 (2017)
11. Brown, D.: Herbal: The Essential Guide to Herbs for Living. Pavilion Books, London (2015)
12. Naghibi, F.; Mosaddegh, M.; Mohammadi Motamed, M.; Ghorbani, A.: Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. Iran. J. Pharm. Res. **4**, 63–79 (2010)
13. Ouakouak, H.; Chohra, M.; Denane, M.: Chemical composition, antioxidant activities of the essential oil of *Mentha pulegium* L., South East of Algeria. Int. Lett. Nat. Sci. **39**, 49–55 (2015)
14. Sardashti, A.; Adhami, Y.: Chemical composition of the essential oil of *Mentha pulegium* L. from Taftan Area by means of gas chromatography/mass spectrometry (GC/MS). J. Med. Plant Res. **7**(40), 3003–3007 (2013)
15. Boukhebti, H.; Chaker, A.N.; Belhadj, H.; Sahli, F.; Ramdhani, M.; Laouer, H.; Harzallah, D.: Chemical composition and antibacterial activity of *Mentha pulegium* L. and *Mentha spicata* L. essential oils. Der. Pharm. Lett. **3**, 267–275 (2011)
16. Teixeira, B.; Marques, A.; Ramos, C.; Batista, I.; Serrano, C.; Matos, O.; Neng, N.R.; Nogueira, J.M.; Saraiva, J.A.; Nunes, M.L.: European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. Ind. Crops Prod. **36**(1), 81–87 (2012)
17. Bouyahya, A.; El Moussaoui, N.; Abrini, J.; Bakri, Y.; Dakka, N.: Determination of phenolic contents, antioxidant and antibacterial activities of strawberry tree (*Arbutus unedo* L.) leaf extracts. Br. Biotechnol. J. **14**, 1–10 (2016)
18. Zaid, A.N.; Jaradat, N.A.; Eid, A.M.; Al Zabadi, H.; Alkaiyat, A.; Darwish, S.A.: Ethnopharmacological survey of home remedies used for treatment of hair and scalp and their methods of preparation in the West Bank-Palestine. BMC Complement Altern. Med. **17**(1), 355–362 (2017)
19. Jaradat, N.A.; Al Zabadi, H.; Rahhal, B.; Hussein, A.M.A.; Mahmoud, J.S.; Mansour, B.; Khasati, A.I.; Issa, A.: The effect of inhalation of Citrus sinensis flowers and Mentha spicata leave essential oils on lung function and exercise performance: a quasi-experimental uncontrolled before-and-after study. J. Int. Soc. Sports Nutr. **13**(1), 36–41 (2016)
20. Jaradat, N.: Quantitative estimations for the volatile oil by using hydrodistillation and microwave accelerated distillation methods from *Ruta graveolens* L. and *Ruta chalepensis* L. leaves from Jerusalem area/Palestine. Mor. J. Chem. **4**(1), 2011–2016 (2016)
21. Vieira, M.; Bessa, L.J.; Martins, M.R.; Arantes, S.; Teixeira, A.P.; Mendes, Â.; Martins da Costa, P.; Belo, A.D.: Chemical composition, antibacterial, antibiofilm and synergistic properties of essential oils from *Eucalyptus globulus* Labill and seven mediterranean aromatic plants. Chem. Biodivers. **14**, 6–10 (2017)
22. Bouyahya, A.; Et-Touys, A.; Bakri, Y.; Talbaui, A.; Fellah, H.; Abrini, J.; Dakka, N.: Chemical composition of *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. Microb. Pathog. **111**, 41–49 (2017)
23. Abdelli, M.; Moghrani, H.; Aboun, A.; Maachi, R.: Algerian *Mentha pulegium* L. leaves essential oil: chemical composition, antimicrobial, insecticidal and antioxidant activities. Ind. Crops Prod. **94**, 197–205 (2016)
24. Shahmohamadi, R.; Sariri, R.; Rasa, M.; Aghamali, M.: Antioxidant activity of gilan *Mentha pulegium* during growth. Pak. J. Biol. Sci. **17**(3), 380–387 (2014)

25. Font-Burgada, J.; Sun, B.; Karin, M.: Obesity and cancer: the oil that feeds the flame. Cell Metabol. **23**(1), 48–62 (2016)
26. Brahmi, F.; Hadj-Ahmed, S.; Zarrouk, A.; Bezine, M.; Nury, T.; Madani, K.; Chibane, M.; Vejux, A.; Andreoletti, P.; Boulekbache-Makhlouf, L.: Evidence of biological activity of Mentha species extracts on apoptotic and autophagic targets on murine RAW264.7 and human U937 monocytic cells. Pharm. Biol. **55**(1), 286–293 (2017)
27. Shirazi, F.H.; Ahmadi, N.; Kamalinejad, M.: Evaluation of northern Iran *Mentha pulegium* L. cytotoxicity. DARU J. Pharm. Sci. **12**(3), 106–110 (2004)
28. Gad-Shayne, C.: Alternatives to In vivo Studies in Toxicology, vol. 1. General and Applied ToxicologyGrove's dictionari's Inc., USA (1999)