JOURNAL OF RADIATION INDUSTRY

Original article

16(4) : 421-427 (2022)
https://doi.org/10.23042/radin.2022.16.4.421

Ionizing Radiation-Inducible delta-3-Carene in Lavender Leaves and Its Anticancer Effect against Various Cancer Cells

Moon-Soo Chung[1], Akhileshwar Singh[1,2], Gyeong Han Jeong[1], Seung Sik Lee[1,2], Byung Yeoup Chung[1] and Sungbeom Lee[1,2,*]

[1]Divison of Radiation Research, Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 56212, Republic of Korea
[2]Department of Radiation Science and Technology, University of Science and Technology, Daejeon 34113, Republic of Korea

Abstract Although essential oil of lavender has many beneficial effects on human health, little is known about its pharmacological effects on cancer cells. Here, we performed solid phase microextraction – gas chromatography – mass spectrometry analysis on volatile terpenoids from lavender (*Lavandula angustifolia* L.) leaves in response to ionizing radiation and identified δ-3-carene, the only monoterpene hydrocarbon increased by γ-irradiation. When lavender leaves were exposed to γ-ray, relative ratio of monoterpene hydrocarbons such as limonene and camphene decreased, but that of δ-3-carene was up-regulated. In pharmacological test, δ-3-carene had suppressive effect on the growth of lung (A549), prostate (PC3 and LNCaP), colon (SW480), breast (MCF7), and liver human cancer cells (Huh7) with an $IC_{50} < 150$ μg mL^{-1} for A549, MCF7, PC3, and LNCaP. Together, δ-3-carene, a γ-ray-inducible volatile in lavender leaves, has inhibitory effects on various cancer cells. These results suggest that δ-3-carene can be a compound of interest as a therapeutic candidate for the study on lung and prostate cancers.

Key words: δ-3-Carene, Terpenes, Lavender, Gamma irradiation, Anticancer effect

1. INTRODUCTION

Terpenoids, also known as isoprenoids, are a class of secondary metabolites in plant that respond to environmental stimuli [1]. While sometimes used interchangeably with "terpenes", terpenoids contain additional functional groups, usually containing oxygen [2]. Terpenes can be classified as mono- (C_{10}), sesqui- (C_{15}), di- (C_{20}), tri- (C_{30}), tetra- (C_{40}), and polyterpenes ($>C_{40}$) according to the number of five-carbon (C_5, isoprene) units that comprise the parent terpene, and their basic backbone structures are synthesized using geranyl diphosphate (GPP), farnesyl diphosphate (FPP), geranylgeranyl diphosphate (GGPP), a head-to-head condensation of FPP or GGPP units as respective substrates [3]. Some terpenoids function as phytoalexins for plant defence against pathogens and herbivores as well as mediators for priming effect which leads to a physiological state that enables plants to respond more rapidly and/or more robustly after exposure to biotic or abiotic stress [4]. For example, limonene (volatile monoterpene) was induced by various abiotic stresses such as γ-ray, UV-B and H_2O_2, and rice bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) was directly suppressed by limonene [1,5]. Terpenoids are also utilized in clinical trials, including anticancer agent taxol (diterpenoid) and anti-malarial drug artemisinin (sesquiterpene lactone) [6,7], and in industrial purposes (e.g., terpenoid-based biofuels and high quality natural rubber (polyterpenoid) [8]).

http://www.ksri.kr/
Copyright © 2022 by
Korean Society of Radiation Industry
***Corresponding author.** Sungbeom Lee
Tel. +82-63-570-3335 Fax. +82-63-570-3339 E-mail. sungbeom@kaeri.re.kr
Received 30 November 2022 **Revised** 3 December 2022 **Accepted** 6 December 2022

Lavender (*Lavandula angustifolia* L.) is known as an aromatic plant for extraction of essential oils, and is a strong-scented perennial herb of Lamiaceae, widely grown in the Mediterranean region [9]. It is used as a spice or condiment widely applied in flavoring foods as well as fragrance, aromatherapy, and pharmaceutical preparations for its therapeutic effects for insomnia and anxiety disorder [10-12]. Also, it is reported that lavender extracts have anticancer effects on human lung cancer cell lines, and two major constituents of lavender essential oil, linalool and linalyl acetate, have antitumor effects on prostate cancer cells [13,14]. Lavender essential oils contain abundant volatile terpene constituents relevant to flavor such as camphor, lavandulyl acetate, terpinen-4-ol, linalyl acetate, and linalool [15,16].

Many researchers have paid attention to increase essential oil content in lavender plants. For example, treatments of biostimulants and hormones, such as FITOSTIM, FITOSTIM ALGA, and jasmonic acid (JA) enhanced the level of essential oil content and terpenoid composition in lavender plants [17,18]. Amino acids-based or seaweed extract-based biostimulants (FITOSTIM and FITOSTIM ALGA) are one of the most employed plant growth promoters [17]. JA was employed as an elicitor aiming to increase the overall content of essential oils by activating secondary metabolites biosynthesis for plant defence [18]. One of brassinosteroid analogues, 24-epibrassinolide, increased plant growth and essential oil content of lavandin (Dutch lavender) [19]. In addition, abiotic responses such as drought stress led to higher production of volatile terpenoids in lavender plants [20]. Although lavender has been investigated on the profiling of volatile compounds under various treatments of biostimulants, hormones, and abiotic stresses, little is known about the compositional change of volatile terpenes in response to ionizing radiation.

Here, we analysed chemical proportional changes of volatile terpenes to identify terpenes whose content increased by γ-irradiation in lavender leaves and identified δ-3-carene as a γ-ray-inducible major hydrocarbon. Further, we examined its anticancer effect on various cancer cell lines including lung, prostate, breast, colon, and liver cancer cells.

2. MATERIALS AND METHODS

2.1. Plant material and γ-irradiation

Two-month-old lavender (*Lavandula angustifolia* L.) plants were obtained from Jeongeup-si lavender farm (Herb ONE, Jeollabuk-do, Republic of Korea, 35°57′21.1″N 126° 88′75.5″E), supported by the Medicinal Resource Research Institute (Jeollabuk-do, Republic of Korea, 35°77′04.4″N 127°37′81.8″E), transferred to fresh potting soil, and grown further for two months in an in-house growth room at 22–24°C, 50–60% relative humidity under a photoperiod of 16-hr light/8-hr dark, and a light intensity of 110–120 μmol m^{-2} s^{-1}. For γ-ray treatments, ten leaves (~500 mg fresh weight) of lavender plants were transferred into a 20-mL glass vial containing 0.5 mL of sterile distilled water, and exposed to 50, 200, 500, and 1,000 Gy for 4 hr using a ^{60}Co source (IR-222; MDS Nordion Inc., Kanata, Canada) in the gamma-irradiation facility located in the Advanced Radiation Technology Institute (ARTI, Jeollabuk-do, Republic of Korea). Lavender leaves without γ-irradiation were used as a control (0 Gy).

2.2. GC–MS analysis of headspace volatile compounds

After 24 hr pre-incubation in the growth room, headspace volatiles from the γ-irradiated lavender leaves were collected for 1 hr using a solid phase microextraction (SPME) fiber coated with polydimethylsiloxane (df 100 μm, needle size 24 Ga; Supelco, PA, USA), followed by immediate injection in a gas chromatography – mass spectrometry (GC–MS) system (GC-7890A/MS-5975C, Agilent, CA, USA). Headspace volatile compounds were separated on a DB-5MS capillary column (0.25 mm i.d. × 30 m length, 0.25 μm film thickness, Agilent) with helium used as the carrier gas and maintained at a flow rate of 1 mL min^{-1}. The oven temperature of GC was set to rise from 80°C (3 min hold) to 240°C (3 min hold) at a rate of 10°C min^{-1} and with a thermal gradient of 20°C min^{-1} up to 300°C (3 min hold). The detailed parameters of the GC–MS instrument were set following conditions: injection temperature, 180°C; transfer line temperature, 280°C; source temperature, 230°C; quadrupole temperature, 150°C; ionization potential, 70 eV; a scan range of 50 to 350 atomic mass units. Terpenoids were identified by using Wiley 12th Edition and National Institute of Standards and Technology (NIST) 2020 Mass Spectral libraries, and the mass spectrometry program version 2.3 (https://chemdata.nist.gov/dokuwiki/).

2.3. Evaluation of volatile terpenes

Abundance of each compound in percentile was estimated

in relative to total peak area of all terpenoids identified. Then, a percentage of each compound was estimated in the average of those from the three plants. Amounts of representative compounds were measured by calibration curves of standard chemicals using limonene (Cat#62128), α-pinene (Cat#80605), camphene (Cat#456055), and δ-3-carene (Cat#21986) in triplicate. The quantification was further corrected by a standard curve of 1-bromodecane (Cat#145785) used as an internal standard. The standard chemicals were purchased from Sigma-Aldrich Co. (MA, USA).

2.4. Cell materials and cell viability assay

Cancer cell lines (A549, PC3, LNCaP, SW480, MCF7, and Huh7) were obtained from the Korean Cell Line Bank (Seoul National University, Seoul, Republic of Korea). Cells were cultured under sterile conditions at 37°C in humidified atmosphere containing 5% CO_2. The cell culture medium consisted of Dulbecco's modified eagle medium (DMEM, Cat#11995073, Gibco, NY, USA) of supplemented with 10% fetal bovine serum (FBS, Cat#16000044, Gibco) and 1% penicillin/streptomycin. Effects of δ-3-carene on cell viability in cancer cells were evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [21]. Cancer cells were seeded at a density of 5×10^4 cells into each well of 96-well plates and incubated for 24 hr at 37°C. The cells were treated with δ-3-carene (0.6, 6, 30, 60, 150, 300, and 600 μg mL^{-1}) which dissolved in free medium and were incubated for 24 hr. After addition of MTT solution (0.5 mg mL^{-1}) to each well, the plates were incubated for 3 hr, and then the produced formazan blue was dissolved in dimethyl sulfoxide (DMSO) after the removal of culture media. Cell viability was determined by the formation of formazan blue using a spectrophotometer and the absorbance was measured at 570 nm. The analysis was performed in triplicate.

2.5. Statistical analyses

Data for evaluation of volatile terpenes and cell viability were analysed by one-way analysis of variance (ANOVA) followed by multiple comparisons using Duncan's multiple range test and Tukey's honestly significant difference test. All statistical analyses were performed in PASW Statistics version 18 software [22].

3. RESULTS AND DISCUSSION

3.1. GC–MS analysis of volatile terpenes in γ-irradiated lavender leaves

In order to identify volatile terpenes whose content is increased by γ-irradiation in lavender leaves, we analysed whether significant alterations in the abundance of volatile terpenes are accompanied by the exposure of γ-ray. Under normal condition (0 Gy), lavender leaves continuously produced a wide array of volatile terpenes, mainly 13 monoterpenes and 5 sesquiterpenes except for terpenes with less than 0.5% content (Fig. 1a). We found that the abundance of δ-3-carene increased when the leaves were exposed to 200 Gy of γ-ray, whereas the abundance of most volatile terpenes was reduced or insignificantly changed by γ-irradiation (Fig. 1b). The δ-3-carene identified in lavender leaves eluted at a retention of 5.23 min, identical to that of the commercially available standard (Fig. 1b). EI mass spectra of the identified δ-3-carene, in the lavender leaves and the commercially available standard, were identical, revealing a base peak at *m/z* 93 and a molecular ion at *m/z* 136, in good agreement with mass spectral library (Fig. 1c).

3.2. Major constituents of non-irradiated lavender leaves

Under normal condition (0 Gy), monoterpene hydrocarbons were major constituents in the headspace volatile terpenes from lavender leaves (~44% of total terpenes), including limonene (27.5%), camphene (4.2%), δ-3-carene (3.5%), and α-pinene (2.3%) (legend for figure in Fig. 1a). Other major compounds were α-santalene (20.3%) and *p*-cymene (15.4%) in respective sesquiterpene hydrocarbon and aromatic monoterpene hydrocarbon (legend for figure in Fig. 1a). According to Grandi *et al.* on chemical profiling of lavender essential oil, most abundant compounds were linalool, linalyl acetate and terpinen-4-ol [16]. These results suggest that composition of terpenes in lavender leaves could be different, depending on environmental conditions for plant growth such as climatic conditions, genotypes, and chemical extraction methods.

3.3. Changes in the content of δ-3-carene after γ-irradiation

In order to confirm quantitative changes of δ-3-carene after γ-ray exposure, we measured the relative proportion and

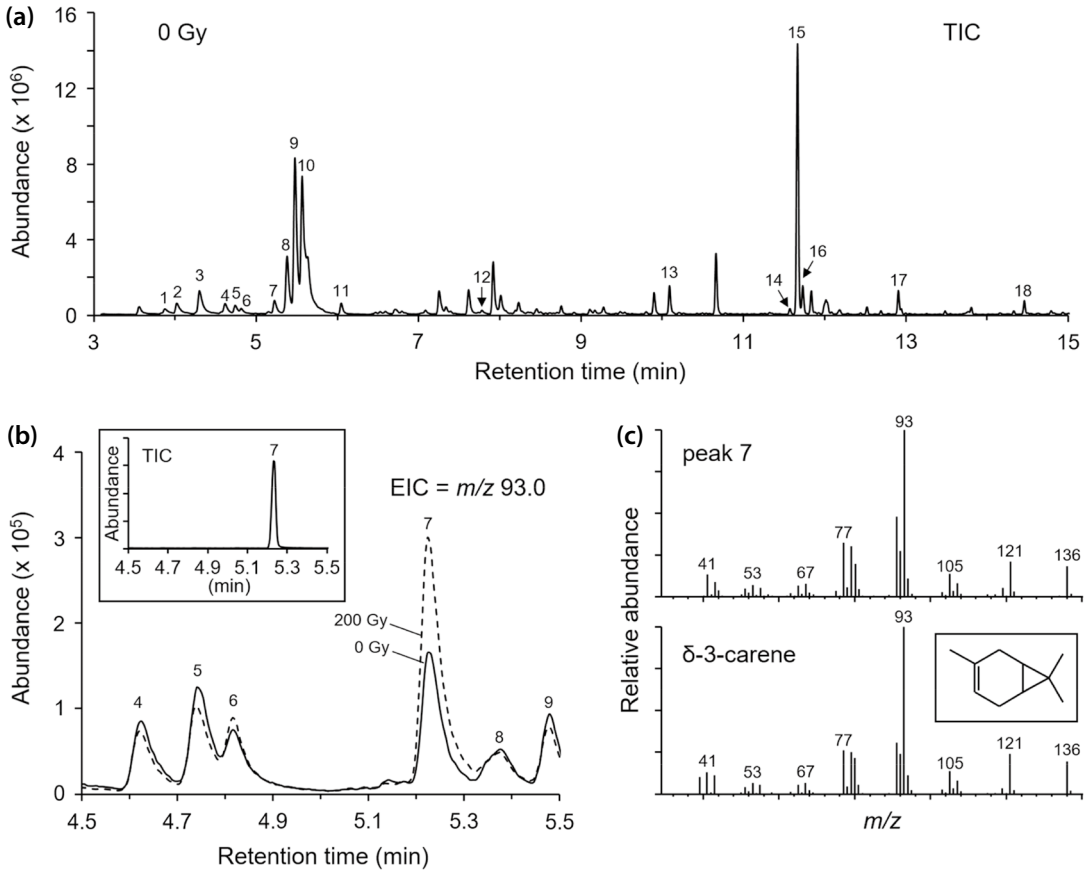

Fig. 1. GC–MS analysis of volatile terpenoids in lavender leaves and identification of δ-3-carene induced by γ-ray. (a) Total ion current (TIC)-generated chromatogram of lavender leaves under normal condition (0 Gy) highlighting its main constituents. The volatile compounds of lavender were identified by GC–MS in the electron positive (EI + ve) mode. A number of terpenes (mono- and sesquiterpenes) were identified and listed as below. 1, α-thujene (1.2%); 2, α-pinene (2.3%); 3, camphene (4.2%); 4, sabinene (1.9%); 5, β-pinene (1.0%); 6, myrcene (1.4%); 7, δ-3-carene (3.5%); 8, *m*-cymene (6.2%); 9, *p*-cymene (15.4%); 10, limonene (27.5%); 11, γ-terpinene (1.0%); 12, camphor (0.7%); 13, eucarvone (1.5%); 14, (*Z*)-α-bergamotene (0.6%); 15, α-santalene (20.3%); 16, (*E*)-α-bergamotene (2.8%); 17, γ-cadinene (1.6%); 18, α-cadinol (0.8%). Each peak area was converted to a percentile in relative to the total peak area of all terpenoids identified. (b) Extracted ion current (EIC, *m/z* 93.0)-generated chromatogram after exposure to 0 or 200 Gy of γ-ray and TIC-generated chromatogram of authentic standard δ-3-carene (inset). (c) EI + ve mass spectrum of δ-3-carene compared with authentic standard and its chemical structure (inset).

amount of δ-3-carene and other monoterpene hydrocarbons (limonene, camphene and α-pinene) produced from lavender leaves after exposure to various doses of γ-irradiation, ranging from 50 to 1,000 Gy. The relative proportion of total monoterpene hydrocarbons declined in a dose-dependent manner from 44% at 0 Gy to 32% at 1,000 Gy (data not shown), including limonene (0.6-fold reduction, 27.5% at 0 Gy → 16.0% at 1,000 Gy), α-pinene (0.6-fold reduction, 2.3% at 0 Gy → 1.3% at 1,000 Gy), and camphene (0.4-fold reduction, 4.2% at 0 Gy → 1.7% at 1,000 Gy), while δ-3-carene (1.9-fold increase, 3.5% at 0 Gy → 6.7% at 1,000 Gy) increased (Fig. 2). The proportion of δ-3-carene mostly increased more than 2.5-fold at 50 Gy (Fig. 2a).

We further performed the quantification of each terpene after γ-irradiation. The amounts of δ-3-carene and limonene were elevated after 200 Gy of γ-irradiation: δ-3-carene (from 0.34 μg at 0 Gy to 0.63 μg at 200 Gy) and limonene (from 2.78 μg at 0 Gy to 3.97 μg at 200 Gy). However, the increment did not follow a dose-dependent manner with 500 Gy and 1,000 Gy of γ-irradiation (Fig. 3a and 3b). On the contrary, the amounts of two monoterpene hydrocarbons significantly decreased under the exposure to γ-ray in a dose-dependent manner: α-pinene (from 0.22 μg at 0 Gy to 0.06 μg at 1,000 Gy) and camphene (from 0.40 μg at 0 Gy to 0.11 μg at 1,000 Gy) (Fig. 3c and 3d). According to previous reports, limonene (volatile monoterpene hydro-

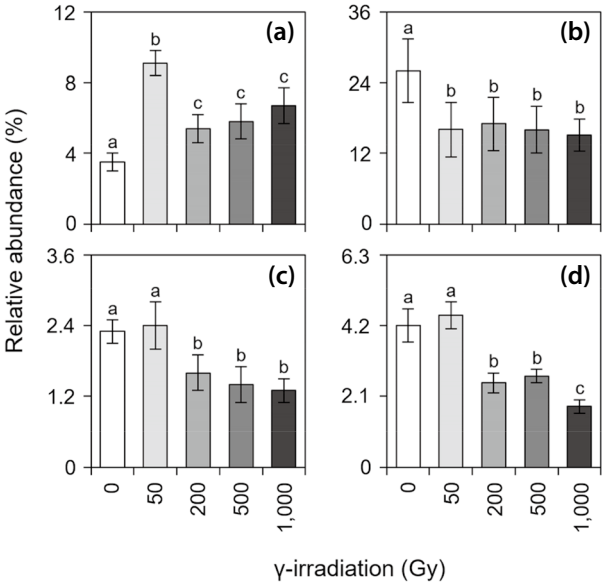

Fig. 2. Relative chemical proportion of representative volatile monoterpene hydrocarbons collected from the lavender leaves after γ-irradiation. Around 10 leaves (~500 mg) of lavender were used for SPME–GC–MS analysis. Each peak area of δ-3-carene (a), limonene (b), α-pinene (c), and camphene (d) was converted to a percentile in relative to the total peak area of all terpenoids identified. Values are means ± SD (n = 3). Letters on top of bars indicate significant differences ($P < 0.05$) by one-way ANOVA with Duncan's multiple range test.

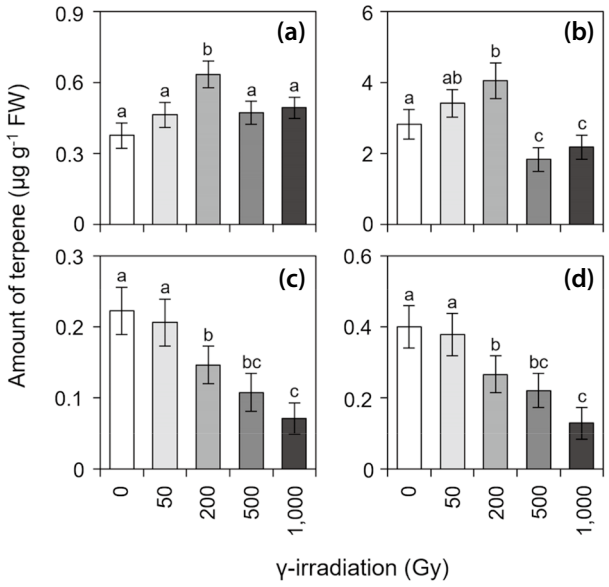

Fig. 3. Quantification of representative volatile monoterpene hydrocarbons induced by γ-irradiation. The amount of δ-3-carene (a), limonene (b), α-pinene (c), and camphene (d) was calculated by calibration curves of authentic standard chemicals. Values are means ± SD (n = 3). Letters on top of bars indicate significant differences ($P < 0.05$) by one-way ANOVA with Duncan's multiple range test. FW, fresh weight.

carbon) was induced by exposure to γ-ray in rice plants and directly inhibited bacterial growth of rice bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) [1,5].

3.4. Anticancer effect of δ-3-carene

Although essential oil of lavender has many beneficial effects on human health, little is known about its pharmacological effects on cancer cells. In order to test the efficacy of δ-3-carene against human cancer cells, we determined the effect of δ-3-carene on cell viability using various cancer cell lines: prostate (PC3 and LNCaP), lung (A549), colon (SW480), breast (MCF7), and liver cancer cells (Huh7). The δ-3-carene significantly inhibited the proliferation of all types of cancer cells at concentrations of 150 μg mL^{-1}: PC3 (27.9% of cell viability, set to 100% at 0 μg mL^{-1}), LNCaP (46.8%), A549 (23.5%), SW480 (61.9%), MCF7 (45.3%), and Huh7 (63.6%) (Fig. 4). The selective cytotoxic activity of δ-3-carene was exhibited against prostate (PC3 and LNCaP), lung (A549), and breast cancer cells (MCF7) with an $IC_{50} < 150$ μg mL^{-1} (Fig. 4a~c and 4e), and δ-3-carene showed more effective inhibition against PC3 and A549 cells (Fig. 4a and 4c). However, the suppressive effect did not follow a dose-dependent manner at low or high concentration of δ-3-carene. As reported previously, the drug (anticancer agent) effect may be essentially independent of concentration when a dose produces near maximal effects [23]. It is suggested that reasonable minimal inhibitory concentration (MIC) and half-maximal inhibitory concentration (IC_{50}) of δ-3-carene is between 60 and 150 μg mL^{-1}. According to Shou-Dong *et al.*, lavender extracts had inhibitory effects on proliferation of human lung carcinoma A549 and hepatoma SMMC7721, and the inhibitory rate is 79.61% and 81.05%, respectively for A549 and SMMC7721 at 48 hr at the concentration of 200 μg mL^{-1} [13]. These results suggest that δ-3-carene can be considered as a putative therapeutic agent in various cancers, and further studies should be performed to understand the cellular and molecular mechanism of δ-3-carene for the anticancer activity.

4. CONCLUSION

In this study, we identified δ-3-carene in lavender leaves in response to gamma irradiation. Although the relative ra-

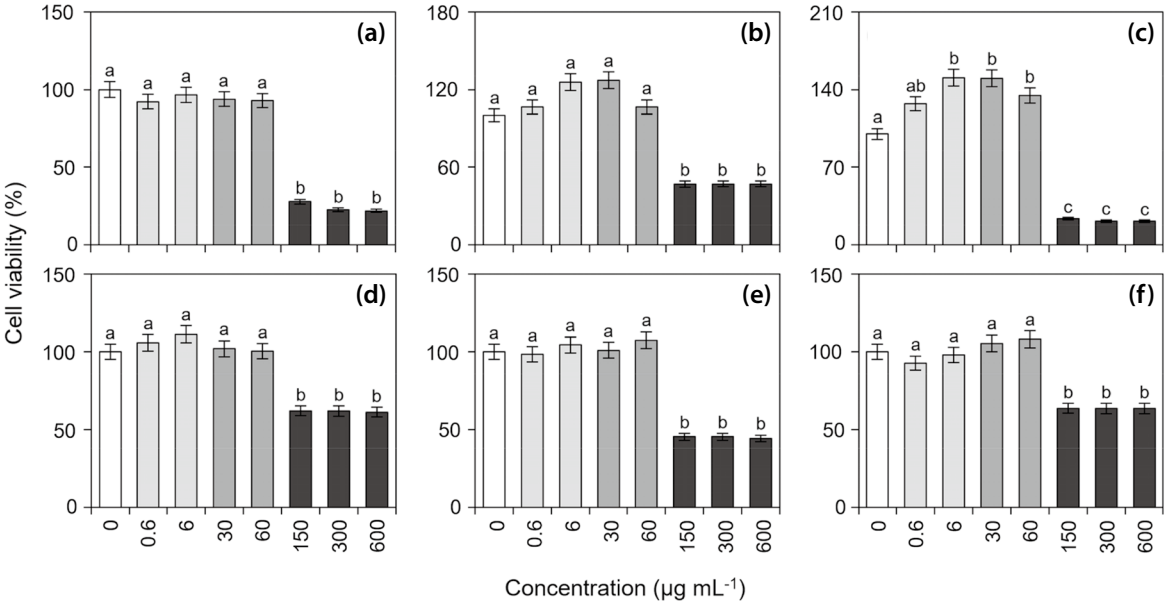

Fig. 4. Effect of δ-3-carene on cell viability in various cancer cell lines. PC3 (a), LNCaP (b), A549 (c), SW480 (d), MCF7 (e), and Huh7 cancer cells (f) were treated with indicated concentrations of δ-3-carene or DMSO (control) for 24 hr. Values are means ± SD (n = 3). Letters on top of bars indicate significant differences ($P < 0.05$) by one-way ANOVA with Tukey's honestly significant difference test.

tio of most monoterpene hydrocarbons decreased by exposure to γ-ray, the ratio of δ-3-carene was up-regulated from 3.5% (0 Gy) to 9.1% (50 Gy) and 6.7% (1,000 Gy). Particularly, the production of δ-3-carene increased more than two folds when the lavender leaves were exposed to 200 Gy of γ-ray, and δ-3-carene had suppressive effects on cell viability against various cancer cell lines. Our results strongly suggest that gamma irradiation can be applied as a tool for non-destructive reconstruction of secondary metabolite profiles in a target plant and the therapeutic property of δ-3-carene has a potential clinical advantage that is widely applicable in various cancers.

ACKNOWLEDGMENTS

This work was supported by the KAERI Institutional Program (Project No. 523220-22) grant funded by the Nuclear R & D Program of the Ministry of Science and ICT (MSIT), Republic of Korea.

REFERENCES

1. Lee GW, Lee S, Chung M-S, Jeong YS and Chung BY. 2015. Rice terpene synthase 20 (OsTPS20) plays an important role in producing terpene volatiles in response to abiotic stresses. *Protoplasma* **252**(4):997-1007. https://doi.org/10.1007/s00709-014-0735-8
2. Moss GP, Smith PAS and Tavernier D. 1995. Glossary of class names of organic compounds and reactivity intermediates based on structure (IUPAC Recommendations 1995). *Pure Appl. Chem.* **67**(8-9):1307-1375. https://doi.org/10.1351/pac199567081307
3. Tholl D. 2006. Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Curr. Opin. Plant Biol.* **9**(3):297-304. https://doi.org/10.1016/j.pbi.2006.03.014
4. Tholl D and Lee S. 2011. Terpene specialized metabolism in *Arabidopsis thaliana*. *Arabidopsis Book* **9**:e0143. https://doi.org/10.1199/tab.0143
5. Lee GW, Chung M-S, Kang M, Chung BY and Lee S. 2016. Direct suppression of a rice bacterial blight (*Xanthomonas oryzae* pv. *oryzae*) by monoterpene (*S*)-limonene. *Protoplasma* **253**(3):683-690. https://doi.org/10.1007/s00709-015-0904-4
6. Croteau R, Ketchum RE, Long RM, Kaspera R and Wildung MR. 2006. Taxol biosynthesis and molecular genetics. *Phytochem. Rev.* **5**(1):75-97. https://doi.org/10.1007/s11101-005-3748-2
7. Pollier J, Moses T and Goossens A. 2011. Combinatorial biosynthesis in plants: a (p)review on its potential and future exploitation. *Nat. Prod. Rep.* **28**(12):1897-1916. https://doi.org/10.1039/c1np00049g
8. Yamashita S, Yamaguchi H, Waki T, Aoki Y, Mizuno M, Yanbe F, Ishii T, Funaki A, Tozawa Y, Miyagi-Inoue Y, Fushihara K, Nakayama T and Takahashi S. 2016. Identification and reconstitution of the rubber biosynthetic machinery on rubber particles from

Hevea brasiliensis. *Elife* **5**:e19022. https://doi.org/10.7554/eLife.19022

9. Allen HD. 2009. Vegetation and ecosystem dynamics. *In*: Woodward J (ed.), The physical geography of the Mediterranean. Oxford University Press, pp. 203-227.
10. Chen TY, Hiyama A, Muramatsu M and Hinotsu A. 2022. The Effect of Lavender on Sleep Quality in Individuals Without Insomnia: A Systematic Review. *Holist. Nurs. Pract*. **36**(4):223-231. https://doi.org/10.1097/hnp.0000000000000528
11. Dakhlaoui S, Wannes WA, Sari H, Hmida MB, Frouja O, Limam H, Tammar S, Bachkouel S, Jemaa MB, Jallouli S, Hessini K and Msaada K. 2022. Combined Effect of Essential Oils from Lavender (*Lavandula officinalis* L.) Aerial Parts and Coriander (*Coriandrum sativum* L.) Seeds on Antioxidant, Anti-diabetic, Anticancer and Anti-inflammatory Activities. *J. Essent. Oil Bear. Plants* **25**(1):188-199. https://doi.org/10.1080/0972060X.2022.2049892
12. Malcolm BJ and Tallian K. 2017. Essential oil of lavender in anxiety disorders: Ready for prime time? *Ment. Health Clin*. **7**(4):147-155. https://doi.org/10.9740/mhc.2017.07.147
13. Shen S-D, Cui C-X, Quan J-S and Shen M-H. 2009. Study on antitumor effect of *Lavender angustifolia* extract. *Food Sci. Technol*. **2**:213-215.
14. Zhao Y, Chen R, Wang Y, Qing C, Wang W and Yang Y. 2017. In Vitro and In Vivo Efficacy Studies of *Lavender angustifolia* Essential Oil and Its Active Constituents on the Proliferation of Human Prostate Cancer. *Integr. Cancer Ther*. **16**(2):215-226. https://doi.org/10.1177/1534735416645408
15. Adaszyńska-Skwirzyńska M and Dzieciol M. 2018. Comparison of Chemical Composition and Antimicrobial Activity of Essential Oils Obtained from Different Cultivars and Morphological Parts of *Lavandula angustifolia*. *J. Essent. Oil Bear. Plants* **21**(6):1532-1541. https://doi.org/10.1080/0972060X.2018.1526123
16. Grandi S, Biffi S, Vecchi A and Barbanti L. 2016. Assessing Essential Oil Composition of Various Lamiaceae Accessions in View of Most Suitable Uses. *J. Essent. Oil Bear. Plants* **19**(6):1351-1367. https://doi.org/10.1080/0972060X.2016.1205958
17. Truzzi E, Benvenuti S, Bertelli D, Francia E and Ronga D. 2021. Effects of Biostimulants on the Chemical Composition of Essential Oil and Hydrosol of Lavandin (*Lavandula x intermedia* Emeric ex Loisel.) Cultivated in Tuscan-Emilian Apennines. *Molecules* **26**(20):6157. https://doi.org/10.3390/molecules26206157
18. Andrys D, Adaszyńska-Skwirzyńska M and Kulpa D. 2018. Jasmonic acid changes the composition of essential oil isolated from narrow-leaved lavender propagated in in vitro cultures. *Nat. Prod. Res*. **32**(7):834-839. https://doi.org/10.1080/14786419.2017.1309533
19. Asci ÖA, Deveci H, Erdeger A, Özdemir KN, Demirci T and Baydar NG. 2019. Brassinosteroids Promote Growth and Secondary Metabolite Production in Lavandin (*Lavandula intermedia* Emeric ex Loisel.). *J. Essent. Oil Bear. Plants* **22**(1):254-263. https://doi.org/10.1080/0972060X.2019.1585298
20. Kumlay AM, Kocak MZ, Gohari G, Nouraein M, Celikcan F, Kaysim MG and Kulak M. 2022. Agronomic traits, secondary metabolites and element concentrations of *Lavandula angustifolia* leaves as a response to single or reiterated drought stress: How effective is the previously experienced stress? *Folia Hortic*. **34**(1):1-16. https://doi.org/10.2478/fhort-2022-0005
21. Carmichael J, DeGraff WG, Gazdar AF, Minna JD and Mitchell JB. 1987. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res*. **47**(4):936-942.
22. Norusis MJ. 2010. PASW Statistics 18 Guide to Data Analysis. Prentice Hall Press, New Jersey.
23. Holford NHG and Sheiner LB. 1981. Understanding the Dose-Effect Relationship. *Clin. Pharmacokinet*. **6**(6):429-453. https://doi.org/10.2165/00003088-198106060-00002