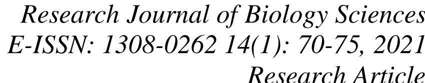

*Research Journal of Biology Sciences*
*E-ISSN: 1308-0262 14(1): 70-75, 2021*
*Research Article*

# INVESTIGATION OF ANTI-CANCER AND ANTIOXIDANT PROPERTIES OF ZINGERONE

Hazal Çobur[1,a,*], Ali Savaş Bülbül[1,b], Sabahattin Cömertpay[2,c]

[1] *Kahramanmaraş Sütçü İmam University, Faculty of Science and Letters, Department of Biology, Kahramanmaraş, Turkey*
[2]*Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Agricultural Biotechnology Division, Kahramanmaraş, Turkey*

**Corresponding Author:*
*E-mail: hazal.cobur@gmail.com*

(Received 10th January 2021; accepted 14th May 2020)

a: ORCID 0000-0002-6464-6893, b: ORCID 0000-0002-2200-7348, c: ORCID 0000-0003-4850-6927

**ABSTRACT.** Zingeron (4-(4-hydroxy-3-methoxyphenyl)-2-butanone) is one of the non-volatile, pungent, phenolic compounds of ginger and this compound is known to have anti-cancer effects. However, comparison of the effect of zingerone on a breast cancer cell line, MCF-7, with that of a healthy cell line, HUVEC (Human Umbilical Vein Endothelial Cells), has been quite limited. In this study; zingerone cytotoxicity on the cells was determined by the MTS test. Accordingly, $IC_{50}$ values for MCF-7 and HUVEC cells were calculated as 2.8 mM and 9.5 mM, respectively. When the values in the two cell groups were compared with the Two-way ANOVA test, it was understood that the p value was less than 0.001. Thus, we concluded that zingerone cytotoxicity on these cells was significantly distinct. The study also examined the DPPH free radical scavenging effect of zingerone and approved that zingerone has antioxidant properties.

**Keywords:** *antioxidant, anti-cancer, MTS, DPPH, in vitro*

## INTRODUCTION

Ginger (*Z. officinale* Roscoe) is a perennial herb belonging to the Zingiberaceae family of the Zingiberales order. It has been traditionally used since ancient times in different parts of the world to help digestion in various human ailments and to treat nausea and vomiting in pregnancy. Due to these medicinal properties, it has played a very important role in primary health care in India and China, and has become one of the most widely used medicinal plants in Ayurveda, China and Japanese medical systems [1, 2, 3].

Ginger represents the source of many bioactive phenolic substances, with pungent non-volatile compounds such as gingerols, shogaols, paradols and zingerone, which create a warm feeling in the mouth. In addition, due to these bioactive components, it has many bioactivities such as antioxidant, anti-inflammatory and antimicrobial properties [4, 5, 6].

Zingerone (4-(4-hydroxy3-methoxyphenyl)-2-butanone) is one of the non-volatile pungent phenolic compounds of ginger. It is structurally closely related to other non-volatile compounds such as gingerol and shogaol, both of which also contain the vanilyl moiety [7, 8].

Breast cancer is the most common type of cancer and the second largest cause of death in women worldwide [9]. The MCF-7 cell line was isolated from a 69-year-old female breast cancer patient in 1970 [10]. It is one of the most widely used cell line because it shows hormone sensitivity through the expression of the estrogen receptor and it is suitable for functional studies of breast cancer cells [11, 12, 13].

Human umbilical vein endothelial cells (HUVEC) have been the most widely used healthy model cells in laboratories as human endothelial cells since 1973 [14]. Because they can be isolated and cultured with minimal requirements [15].

Antioxidants are compounds that protect against oxidative stress caused by free radicals, which have serious damage to healthy cells in the body. Antioxidants accomplish this protection through reactions that occur by interacting with free radicals without damaging other healthy cells [16].

The aim of this study is to compare the cytotoxic activities of zingerone on MCF-7 and HUVEC cell lines and to examine the antioxidant capacity of this compound.

## MATERIALS AND METHODS

### *Cell Culture*

Michigan Cancer Foundation-7 (MCF-7) human breast cancer cell lines were purchased from the Turkish FMD Institute. Human umbilical vein endothelial cells (HUVEC) healthy cell lines were obtained from Agricultural Biotechonology Laboratory of Kahramanmaraş Sütçü İmam University. MCF-7 cells were grown in 10% FBS and 1 % penicillin-streptomycin supplemented RPMI 1640 while DMEM was used instead of RPMI in the same formulation for HUVEC. The cells were maintained in a humid environment at 37 °C with 5% $CO_2$.

### *Zingerone*

The product catalog number W312401 was purchased from Sigma-Aldrich (Germany). 77.68 mg of this ≥ 96% pure substance was dissolved in 100 µL of absolute ethanol to prepare a 4 M concentration of zingerone stock. The stock was stored at -20 °C. Working solutions were obtained by diluting this stock in medium. All working solutions were prepared fresh on the day of the study.

### *Cell Viability Test*

For this test, the protocol called MTS Assay was applied as recommended by the company from which the MTS agent (CellTiter 96® AQueous Assay; Promega, USA) was purchased. In addition, zingerone application on cells was carried out by making necessary changes in the method of [17]. Briefly, cells seeded with 5000 cells in each well of the 96-well plate were exposed to zingerone at various concentrations (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 mM) for 24 hours. At the end of the period, 10 µL of MTS was added to these wells and absorbances at 490 nm were measured after 3-4 hours of incubation at 37°C. With the calculations made on these absorbance values, graphs were drawn on Graph Pad Prism and $IC_{50}$ values were calculated.

### *Determination of DPPH Free Radical Removal*

To determine the antioxidant capacity of zingerone, DPPH (2,2-diphenyl-1-picrylhydrazil) agent and BHT (2,6-Di-tert-butyl-4-methylphenol) were used as control. DPPH, BHT and zingerone (0.01, 0.1, 1, 10 mM) were studied at 4 different concentrations. DPPH and zingerone were dissolved in ethanol. 900 µL of DPPH and 100 µL of zingerone solution were added from the concentrations prepared in 4 replications in a 96-well plate. After treatment with Zingerone, it was incubated at room temperature for 30 minutes. At the end of this period, DPPH Free Radical Removal values of

zingerone were obtained with the help of a spectrophotometer device at a wavelength of 517 nm. DPPH free radical scavenging capacity (%) was calculated with the help of the formula given below;

% DPPH Activity=[Control Absorbance-Sample Absorbance/Control Absorbance] x 100

**Eqn. 1**

### *Statistical Analysis*

In the anticancer phase of our study, the data on the cytotoxic effects of zingerone on MCF-7 and HUVEC cell lines were statistically analyzed using the program called GraphPad Prism. With the program we used, the IC50 value we obtained for both cell lines was calculated. In addition, in order to compare the cytotoxic effect of zingerone on two cell lines, we performed a two-way ANOVA test with the GraphPad Prism program and reported the statistical difference between them.

## RESULTS AND DISCUSSION

### *Zingerone MTS Test Results*

**Fig. 1** shows the analysis of cell viability in MCF-7 cells with various concentrations of zingerone (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 mM) over 24 hours by the MTS viability assay. The $IC_{50}$ value of Zingerone was determined to be 2.8 mM for MCF-7 cells.

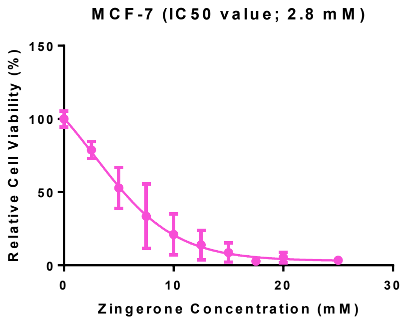


*Fig. 1. Cytotoxic effect of Zingerone on MCF-7 cells*

**Fig. 2** shows analysis of cell viability in HUVEC cells with various concentrations of zingerone (0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, 20, 25 mM) over 24 hours with the MTS viability assay. The $IC_{50}$ value of Zingerone was determined to be 9.5 mM for HUVEC cells.

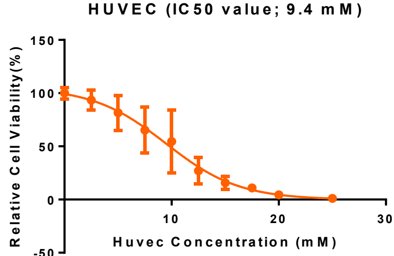


*Fig. 2. Cytotoxic effect of Zingerone on HUVEC cells*

**Fig. 3** shows the statistical analysis of the study performed by the MTS viability assay of zingerone compared to MCF-7 on HUVEC cells.

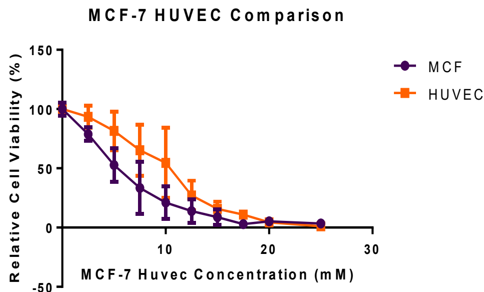


*Fig. 3. Comparative graphic of the cytotoxic effect of Zingerone on MCF-7 and HUVEC cells*

### DPPH Free Radical Scavenging Capacity Results of Zingerone

**Fig. 4**, as a result of the determination of the antioxidant capacity of zingerone with the DPPH test, when the free radical scavenging capacities of BHT and zingerone (%) used as the control group were compared, it was found that the antioxidant value of Zingerone was significantly higher than BHT at 1 mM and 10 mM concentration.

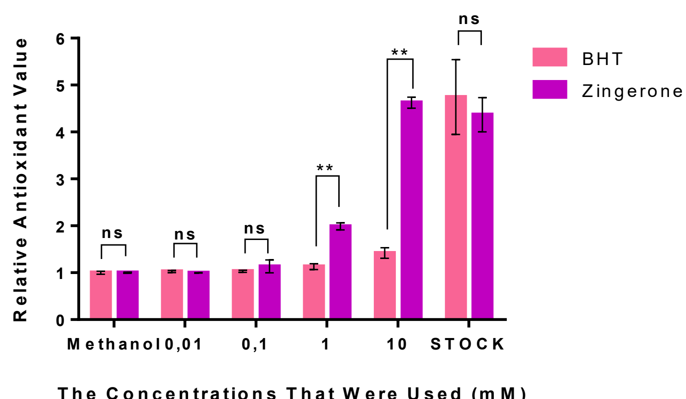


*Fig. 4. DPPH Free Radical Scavenging values of Zingerone*

In this thesis we have carried out, the cytotoxic effects of Zingeron (4-(4-hydroxy-3-methoxyphenyl)-2-butanone), one of the non-volatile pungent phenolic compounds of ginger, on breast cancer (MCF-). 7) cell line and healthy cell (HUVEC) cell line were compared and antioxidant capacity was determined.

Consequently; The $IC_{50}$ value of Zingerone on the MCF-7 breast cancer cell line was determined as 2.8, while the IC50 value on the HUVEC cell line was determined as 9.4. Zingerone has been found to have a cytotoxic effect on both cell lines.

However, the $IC_{50}$ value that it showed on the MCF-7 cell line was lower than the HUVEC cell line ($p<0.001$).

Supporting our study, Gan et al. [18] also reported the anticancer properties of zingerone (ZO). However, the $IC_{50}$ value they calculared was different (15 µM ). The difference of the values might be caused by the fact that they used DMEM to grow the

cells, they picked MTT instead of MTS for vaibility assessment and they measured the absorbances at 650 nm and 550 nm while ours was 490 nm.

Manjunath et al. [19] evaluated the radical scavenging activities (RSA) of tetrahydrocurcumin (THC), zingerone and quinoline derivatives in their study. In this study, they stated that zingerone showed antioxidant activity. Determining the antioxidant capacity of zingerone made with DPPH, and methanol used to dissolve DPPH are similar to its inclusion in the control group as in our study.

## CONCLUSION

Our study was carried out using only one model cell line for breast cancer and a healthy cell population. In order to reach a solid conclusion, more studies with different cancer lines and healthy cells must be conducted.

## REFERENCES

[1] Nigam, N., George, J., Shukla, Y. (2009): Ginger (6-gingerol). Molecular targets and therapeutic uses of spices: modern uses for ancient medicine. (Eds: Aggarwal, B. and Kunnumakkara, A.). Singapore: World Scientific.

[2] Murray, M. T., and Pizzorno, J. (2013): Immune support. Textbook of Natural Medicine, 4th Edition. St. Louis: Churchill Livingstone.

[3] Nair, K. P. (2019): Turmeric (*Curcuma longa* L.) and Ginger (*Zingiber officinale* Rosc.)- World's Invaluable Medicinal Spices: The Agronomy and Economy of Turmeric and Ginger. Springer Nature.

[4] Srinivasan, K. (2017): Ginger rhizomes (*Zingiber officinale*): A spice with multiple health beneficial potentials. Pharma Nutrition 5: 18-28.

[5] Dhanik, J., Arya, N., Nand, V. (2017): A review on *Zingiber officinale*. Journal of Pharmacognosy and phytochemistry 6(3): 174-184.

[6] Mao, Q. Q., Xu, X. Y., Cao, S. Y., Gan, R. Y., Corke, H., Li, H. B. (2019): Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). Foods 8(6): 185.

[7] Nagabhushan, M., Amonkar, A., Bhide, S. (1987): Mutagenicity of gingerol and shogaol and antimutagenicity of zingerone in Salmonella/microsome assay. Cancer letters 36(2): 221-233.

[8] Ahmad, B., Rehman, M. U., Amin, I., Arif, A., Rasool, S., Bhat, S. A., Afzal, I., Hussain, I., Bilal, S., Mir, M. (2015): A review on pharmacological properties of zingerone (4-(4-Hydroxy-3-methoxyphenyl)-2-butanone). The Scientific World Journal.

[9] Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R., Torre, L., Jemal, A. (2018): Global Cancer Statistics 2018: Globocan Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: a cancer journal for clinicians 68(6): 394-424.

[10] Soule, H., Vazquez, J., Long, A., Albert, S., Brennan, M. (1973): A human cell line from a pleural effusion derived from a breast carcinoma. Journal of the national cancer institute 51(5): 1409-1416.

[11] Osborne, C., Hobbs, K., Trent, J. (1987): Biological differences among MCF-7 human breast cancer cell lines from different laboratories. Breast cancer research and treatment 9(2): 111-121.

[12] Levenson, A. S., Jordan, V. C. (1997): MCF-7: the first hormone-responsive breast cancer cell line. Cancer research 57(15): 3071-3078.

[13] Comşa, Ş., Cimpean, A. M., Raica, M. (2015): The story of MCF-7 breast cancer cell line: 40 years of experience in research. Anticancer research 35(6): 3147-3154.

[14] Jaffe,, E., Nachman, R., Becker, C., Minick, C. (1973): Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. The Journal of clinical investigation 52(11): 2745-2756.

[15] Kocherova, I., Bryja, A., Mozdziak, P., Volponi, A., Dyszkiewicz-Konwińska, M., Piotrowska-Kempisty, H., . . . Kempisty, B. (2009). Human umbilical vein endothelial cells (HUVECs) co-culture with osteogenic cells: from molecular communication to engineering prevascularised bone grafts. Journal of clinical medicine 8(10): 1602.

[16] Lü, J.-M., Lin, P., Yao, Q., Chen, C. (2010): Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. Journal of cellular and molecular medicine 14(4): 840-860.

[17] Cömertpay, S., Demirbanka, F. G. Ö. (2020): Lowered Cyclin E levels increase the efficiency and the specificity of capsaicin against cancerous cells of mesothelium. Cellular and Molecular Biology 66(6): 98-104.

[18] Gan, H., Zhang, Y., Zhou, Q., Zheng, L., Xie, X., Veeraraghavan, V., Mohan, S. (2019): Zingerone induced caspase-dependent apoptosis in MCF-7 cells and prevents 7, 12-dimethylbenz (a) anthracene-induced mammary carcinogenesis in experimental rats. Journal of biochemical and molecular toxicology 33(10): e22387.

[19] Manjunatha, J., Bettadaiah, B., Negi, P., Srinivas, P. (2013): Synthesis of quinoline derivatives of tetrahydrocurcumin and zingerone and evaluation of their antioxidant and antibacterial attributes. Food chemistry 136(2): 650-658.