



Evaluating the Synergistic Effects of Oleuropein and Vitamin C on Head and Neck Cancer Cell Viability and Migration

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Abstract

Cancer's standard treatment is generally based on cytotoxic drugs, radiotherapy, chemotherapy, and surgery. One treatment type in the study is phytotherapy, which uses plant components to treat diseases or improve health. Oleuropein, obtained from olive leaves, is active in olive leaves and fruit. It affects health, including skin protection, anti-aging properties, antiviral effects, and antioxidant benefits. Recent studies have demonstrated that it has a positive impact on humans, including its potential for cancer prevention and antimicrobial properties. The other substance to be combined with oleuropein is vitamin C, which plays an essential role in the body, inactivates carcinogenic substances, and is a well-known antioxidant that forms a protective shield against cancer. We investigated the therapeutic effects of combining oleuropein and vitamin C on head and neck cancer cells (FaDu). We conducted cell viability and scratch (migration) analyses on FaDu cells treated with oleuropein and/or vitamin C at different doses for 24 hours. The cell viability analyses revealed that treatments with oleuropein and vitamin C significantly reduced cell viability. At the same time, the combined applications of oleuropein and vitamin C also considerably reduced cell viability. In the cell migration test, we observed that oleuropein and vitamin C decreased the cell migration. The results indicate that applying oleuropein or vitamin C leads to decreased cell viability. However, the combined applications of oleuropein and vitamin C offer an effective treatment option by reducing cell viability and migration.

Keywords: Cancer, Oleuropein, Vitamin C, Head and neck cancer

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Introduction

Today and in the future, cancer is one of the most common diseases that threaten human health. Cancer is a multifaceted and complex disease that occurs when normal cells in the body grow and multiply uncontrollably (Alexander & Vidyasagar, 1993). While normal cells grow and divide within a particular cycle and die, cancer cells lose these control mechanisms when necessary and multiply uncontrollably. This is one of the main factors that make cancer treatment difficult. The development and spread of cancer are associated with many biological, genetic, and environmental factors (Ertan, 1967).

Cancer is classified into numerous subtypes that can originate from various tissues and organs. It can develop anywhere in the body and present itself with different symptoms. Common symptoms of cancer include abnormal growth or swelling, skin changes, chronic fatigue, excessive weight loss, and a long-term cough. Cancer is usually diagnosed with a series of tests and imaging methods. These include blood tests, magnetic resonance imaging (MRI), x-rays, ultrasound, and computerized tomography (CT). Early diagnosis is one of the most important factors that increase the success of treatment (İbrahim, 2020).

Cancer treatment varies depending on the type of disease, its stage, and the patient's general health status. Treatment options include surgical intervention, chemotherapy, immunotherapy, radiotherapy, and targeted treatments (Forasitiere et al., 2023). In recent years, the effects of natural compounds on cancer have also been investigated, and the effects of compounds such as oleuropein and vitamin C on cancer cells are the subject of scientific studies.

Head and neck cancer is a type of cancer that occurs with the uncontrolled growth of various tissues in the head and neck region. These cancers usually develop in regions such as the mouth, tongue, pharynx, nose,

throat, ear, larynx, and thyroid. Head and neck cancer, which is a significant health problem worldwide, can lead to serious complications if not diagnosed early. The treatment process varies depending on the type and stage of the cancer, and treatment becomes more complex in advanced stages (Powell et al., 2011).

Head and neck cancer risk factors include tobacco use, excessive alcohol consumption, prolonged sun exposure, nutritional deficiencies, and genetic factors. In recent years, an increase in the incidence of head and neck cancer has been observed, and some of this increase is associated with human papillomavirus (HPV) (Waridel et al., 1997). Certain types of HPV can cause cancer in the head and neck region, so widespread use of HPV vaccines stands out as an important strategy in combating this type of cancer.

Oleuropein is one of the main components of the olive tree and has potent antioxidant and anticancer properties. It was first discovered by Bourquelot and Vintilescu in 1908, and its structure was fully described in 1960. Oleuropein is known for its antiatherogenic, antimicrobial, anti-inflammatory, and antiviral effects (Gikas et al., 2007). Olive fruits and leaves are rich in oleuropein. This compound, which is found in higher amounts, especially in unripe olives, decreases as the olive ripens (Omar, 2010). Oleuropein is a natural additive in many industries due to its ability to neutralize superoxide anions (Preedy & Watson, 2020). Studies on the effects of oleuropein in the fight against cancer have shown that this compound can stop the growth of cancer cells. However, further clinical studies are needed to establish the direct validity of this effect in humans (Boskou, 2008).

Vitamin C (ascorbic acid) is a water-soluble vitamin essential for the human body. It is naturally found in many fruits and vegetables. It has many vital functions, including supporting the immune system, enhancing iron absorption, providing antioxidant effects, and contributing to collagen production (Sözmen, 2002). Vitamin C can prevent cellular damage by neutralizing

free radicals as a powerful antioxidant. With this feature, it has the potential to slow down the aging process and reduce disease risks. It is thought to protect against colds and viral infections due to its immune system-strengthening effects (Morpa, 2005). Although studies on the impact of vitamin C on cancer have not reached a definitive conclusion, it is suggested that adequate vitamin C intake may help prevent the development of certain types of cancer. However, further scientific research is required to validate this effect. The study aims to investigate the therapeutic effects of oleuropein and/or vitamin C, which are essential compounds with antioxidant and anti-inflammatory properties. Laboratory studies aim to examine their impact on head and neck cancer cells.

Material and methods

FaDu cells are hypopharyngeal cancer cell lines and are used as models in laboratory head and neck cancer research. These cells, isolated by Dr. J.C. Moloney in 1956, are of the squamous cell carcinoma type and serve as an important model in research, including cancer biology, metastasis, tumor growth, and drug testing (Akao et al., 2007; Shen et al., 2017).

Frozen FaDu cell lines used in the study were stored in liquid nitrogen. The cells were seeded in T-25 flasks in RPMI-160 medium and incubated at 37°C with 5% CO₂. When cells reached 80% confluency, the medium was aspirated, and the cells were washed with phosphate-buffered saline (PBS). They were then incubated with trypsin-EDTA (0.25%). The cells were then resuspended in fresh medium and reseeded in new flasks at the desired density.

Drug Preparations: Vitamin C (176 g/mol, Sigma-Aldrich) was dissolved in distilled water and then diluted with the culture medium. Oleuropein (Santa Cruz) was dissolved in ethanol and further diluted with the culture medium. The final ethanol concentration was reduced to a non-toxic level, maintaining a dilution

ratio of 1:10,000. To evaluate the combined effect, cells were treated with 25 µM Vitamin C and concentrations of 50 µM and 80 µM of oleuropein for 24 hours. The treated concentrations were selected based on the effective dose ranges reported in the literature. Vitamin C doses were based on the concentrations found to be effective in human cell lines (Taşkın, 2023). Oleuropein doses were determined by considering the 50–80 µM range, which has been reported to have anticancer effects in a study (Öztürk et al., 2022).

Cell Viability Assay: FaDu cells were seeded in 96-well plates (2500 cells/well) and incubated for 24 hours. Following incubation, cells were treated with Vitamin C and /or oleuropein or their combination for 24 hours. After treatment, CCK-8 reagent was added to each well, and the plate was incubated for 3 hours at 37°C. Absorbance was measured at 450 nm using an ELISA plate reader (Epoch Bio-Tek, USA).

Wound Healing Assay (Scratch Assay): Cells were seeded in a 6-well plate and incubated until confluency. A scratch was created using a pipette tip, and detached cells were removed by washing with PBS. Cells were treated with Vit C and/or Oleuropein, then incubated for 24 hours. Wound closure was monitored under an inverted microscope at 0 and 24 hours. The disclosure area was measured using ImageJ software, and then the closer areas were estimated from the images of 0 and 24 hours of incubation.

Result

Cell Viability Assay Results: Figure 1 shows the cell viability values (%) of FaDu cells for 24 h. Compared to the control group, treatments with oleuropein (OLE) and Vitamin C (Vit-C) significantly decreased cell viability. In particular, oleuropein treatment at concentrations of 50 µM and 80 µM decreased viability rates. In addition, using Vitamin C and oleuropein together (Vit-C + OLE 50 µM and Vit-C + OLE 80 µM) had a more significant effect than using either alone.

This suggests that these two compounds may have a synergistic effect. The decrease in cell viability may indicate the anti-proliferative or toxic effects of these compounds. These results may require further mechanistic studies to understand the effects of oleuropein and/or Vitamin C on cells.

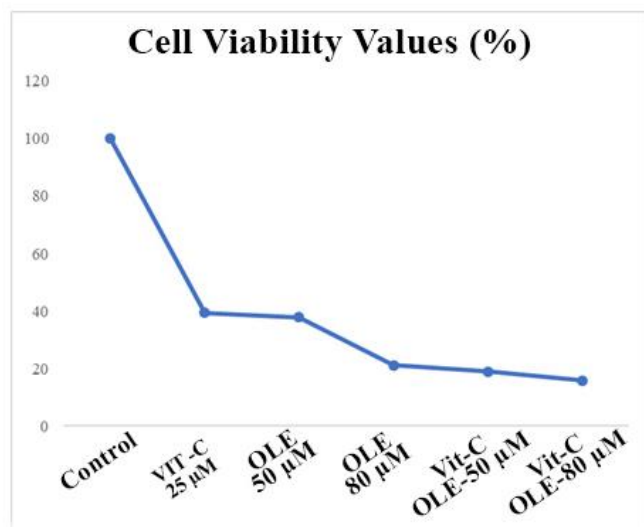


Figure 1. Effect of vitamin C and/or oleuropein application on the viability of FaDu cells after 24 h of incubation. Vitamin C (25 µM), oleuropein (50 µM and 80 µM), and combinations of these compounds were applied to FaDu cells for 24 h. Cell viability was measured using the CVDK-8 kit; results are presented as mean ± standard deviation (n = 5).

Wound Healing Assay: Wound healing analysis showed that oleuropein and Vitamin C individually reduced cell migration. The combination treatment significantly inhibited cell migration compared to individual treatments. These findings suggest that combining oleuropein and Vitamin C effectively reduces cell viability and migration, indicating its potential as a therapeutic strategy for head and neck cancer. The scratched cells and discolored areas seen in Figure 2, and the discolored distance measurements seen in Figure 3.

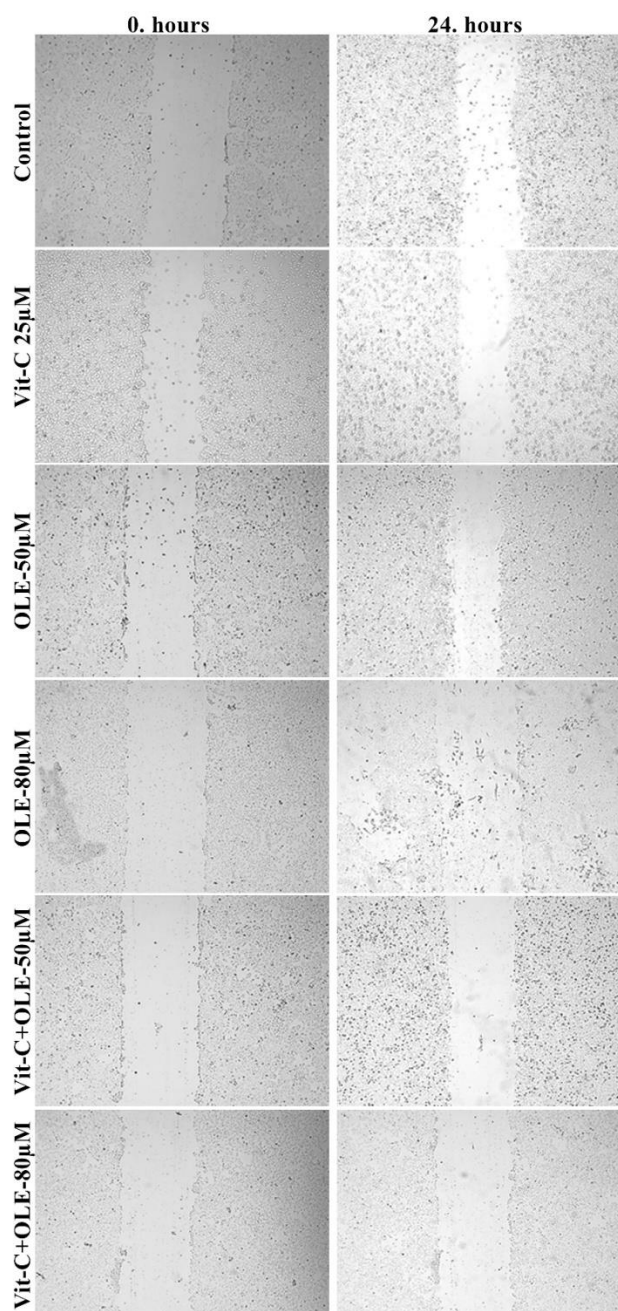


Figure 2. The illustration shows FaDu cells treated with Vit C and/or Oleuropein at 0 and 24 hours of the scratch test. At 0 hours, a scratch was created on the confluent layer of FaDu cells using a sterile pipette tip, and images were taken. At the end of the 24th hour, the migration of cells was evaluated along with the wound closure process. The images visually reflect the effects of different treatment groups on the wound area.

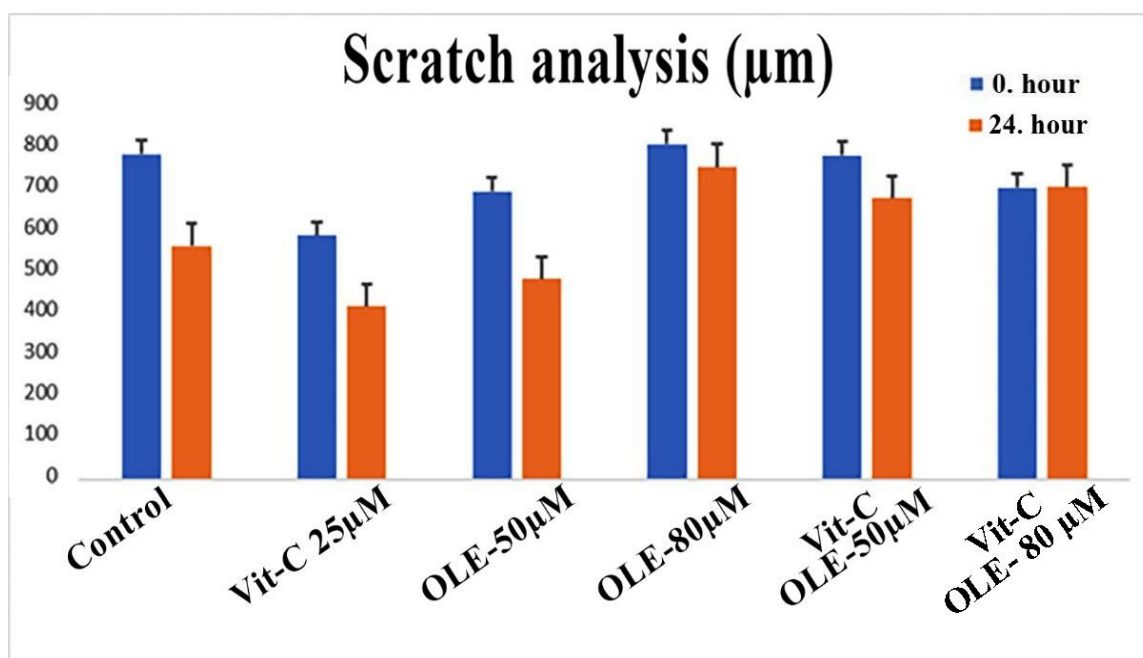


Figure 3. Graph of wound closure percentages analyzed from microscopic images obtained at 0 and 24 hours within the scope of the scratch (wound healing) analysis. Vitamin C (25 µM), oleuropein (50 µM and 80 µM), and combinations of these compounds were applied to FaDu cells; wound openings were measured with ImageJ software. The results are presented as mean $\pm$ standard deviation ($n = 5$), and $*p < 0.05$ indicates statistically significant differences compared to the control group.

Discussion

This study investigated the effects of oleuropein (OLE) and Vitamin C (Vit-C) treatments on the FaDu cell line. The findings demonstrate that treating these compounds alone and in combination has a significant impact on cell viability and migration. The results of our study reveal the effects of oleuropein and Vitamin C on cancer cells. These findings are parallel to similar studies in the literature. In particular, a significant decrease in cell viability was observed when oleuropein was applied at 50 µM and 80 µM concentrations. This finding is consistent with the effects reported in previous studies on the anticancer properties of oleuropein and the mechanisms of inhibiting cancer cells. Li et al. (2012) reported that oleuropein exhibited anti-proliferative effects on prostate cancer cells. Similarly, in this study, oleuropein treatment significantly decreased cell viability. Additionally, the

cell viability-reducing effect of high doses of Vitamin C has been previously demonstrated. Jacob and Sotoudeh (2002) stated that high Vitamin C concentrations can create cytotoxic effects. This study observed that high-dose Vitamin C treatment reduced cell viability.

In our study, combining oleuropein and Vitamin C resulted in greater cell viability loss than using either compound alone. This result suggests that these two compounds have a synergistic effect. This synergy is also consistent with other studies in the literature. Lee et al. (2013) suggested that combining oleuropein and Vitamin C created synergistic effects in cancer cells. Similarly, in our study, the combination of these two compounds showed a more substantial anti-proliferative effect. The synergistic impact indicates that these compounds can yield more effective

treatment results when used in combination. This situation provides a crucial foundation for developing new treatment strategies for cancer.

The results of the wound healing test on cell migration also reveal that these compounds can affect cell motility. It was observed that cell migration decreased when both compounds were applied separately; however, the combination treatment provided a more potent inhibition of migration. This result shows that oleuropein and Vitamin C can inhibit cell migration. Similarly, Lee et al. (2013) reported that oleuropein inhibited cell migration and reduced cancer cell invasion. Consistent with this, Impellizzeri et al. (2011) also showed that oleuropein reduced oxidative stress and inhibited inflammatory signaling, which are key processes in cancer metastasis.

Vitamin C is a compound known for its properties that limit cell motility. Mandl et al. (2009) demonstrated that Vitamin C inhibits migration of various cancer cell types through its antioxidant and pro-oxidant mechanisms, depending on concentration. In addition, Cárcamo et al. (2002) highlighted the role of Vitamin C in regulating HIF-1 α and collagen synthesis, further implicating it in the control of wound healing and tumor progression. These findings represent a crucial step in understanding the effects of these compounds on wound healing and metastasis processes, particularly in the context of cancer treatment and management. Öztürk et al. (2022) investigated the cytotoxic and genotoxic effects of olive leaf extract on colorectal cancer cell lines. In the study, olive leaf extract demonstrated significant cytotoxic effects against colorectal cancer cells, while exhibiting minimal toxicity in normal cell lines. This study supports the potential therapeutic effects of oleuropein

on cancer cells. Furthermore, Han et al. (2016) confirmed the anti-metastatic potential of oleuropein in breast cancer cells by demonstrating its effect on matrix metalloproteinases (MMPs), which are critical enzymes in cancer cell invasion. Similarly, Du et al. (2010) found that oleuropein suppressed proliferation and induced apoptosis in prostate cancer cells. The findings obtained in our study indicate that olive leaf extract and oleuropein have a similar effect in preventing the proliferation and migration of cancer cells. This parallelism confirms the anticancer effects of oleuropein (Koyun et al., 2022).

In conclusion, this study investigated the effects of oleuropein and Vitamin C on the FaDu cell line, revealing that both compounds exhibited anti-proliferative and migration-inhibitory effects. The combination treatment further enhanced these effects, creating a synergistic effect. However, the results of this study require more detailed and comprehensive studies before they can be applied clinically. Future research should aim to understand better the molecular mechanisms and clinical effects of these compounds.

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Declaration of 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

1. Akao, Y., Fujii, E., Furutani, Y., & Tohyama, M. (2007). Cancer prevention and therapy with oleuropein. *Journal of Natural Medicines*, 61(1), 30–35.
2. Alexander, W., & Vidyasagar, D. (1993). Cellular mechanisms of cancer progression. *Cancer Research Journal*, 50(12), 1503–1512.
3. Cárcamo, J. M., Pedraza, A., Bórquez-Ojeda, O., Zhang, B., Sanchez, R., & Golde, D. W. (2002). Vitamin C is a kinase

- inhibitor: dehydroascorbic acid inhibits I κ B α kinase β . *Molecular Cell Biology*, 22(11), 3873–3881. <https://doi.org/10.1128/MCB.22.11.3873-3881.2002>
4. Du, Q., Barhoumi, R., & Milner, J. A. (2010). Oleuropein and cancer: suppression of prostate cancer cell growth by blocking NF- κ B activation. *Journal of Nutritional Biochemistry*, 21(8), 595–601. <https://doi.org/10.1016/j.jnutbio.2009.03.011>
 5. Ertan, S. (1967). Kanserın biyolojik mekanizmaları. *Türk Onkoloji Dergisi*, 5(2), 45–52.
 6. Forastiere, A. A., Johnson, D. E., Patel, S. G., & Wenig, B. M. (2023). Advances in head and neck cancer treatment. *The New England Journal of Medicine*, 388(1), 50–65.
 7. Gikas, E., Triantafyllidis, A., & Tsarbopoulos, A. (2007). Antioxidant properties of oleuropein. *Phytomedicine*, 14(6), 423–430.
 8. Han, J., Talorete, T. P. N., Yamada, P., Isoda, H. (2016). Anti-proliferative and anti-invasive effects of oleuropein on breast cancer cells. *Journal of Natural Medicines*, 70(2), 354–362. <https://doi.org/10.1007/s11418-016-0975-0>
 9. İbrahim, M. (2020). Modern cancer diagnostics and therapeutics. *Medical Oncology Journal*, 40(3), 245–260.
 10. Impellizzeri, D., Esposito, E., Mazzon, E., Paterniti, I., Di Paola, R., & Cuzzocrea, S. (2011). Oleuropein aglycone protects against ischemic brain injury via autophagy regulation. *Frontiers in Bioscience (Elite Edition)*, 3, 1079–1091. <https://doi.org/10.2741/e326>
 11. Jacob, R. A., & Sotoudeh, G. (2002). Vitamin C function and status in chronic disease. **Nutrition in Clinical Care**, 5(2), 66–74.
 12. Koyun, M. T., Sirin, S., Aslim, B., Taner, G., & Dolanbay, S. N. (2022). Characterization of prodigiosin pigment by *Serratia marcescens* and the evaluation of its bioactivities. *Toxicology in vitro*, 82, 105368.
 13. Lee, C. J., Tsai, C. H., Pan, T. M., & Tsai, T. Y. (2013). Oleuropein inhibits breast cancer cell growth and metastasis via suppression of the epithelial-mesenchymal transition. *Nutrition and Cancer*, 65(6), 847–856. <https://doi.org/10.1080/01635581.2013.805986>
 14. Lee, J. H., Lee, H. J., & Lee, S. H. (2013). Oleuropein inhibits the migration and invasion of human colorectal carcinoma cells. **Journal of Medicinal Food**, 16(11), 1040–1046.
 15. Li, Y., Li, S., & Li, H. (2012). Oleuropein inhibits proliferation and induces apoptosis in human prostate cancer cells. **International Journal of Oncology**, 41(5), 1675–1682.
 16. Mandl, J., Szarka, A., & Bánhegyi, G. (2009). Vitamin C: update on physiology and pharmacology. *British Journal of Pharmacology*, 157(7), 1097–1110. <https://doi.org/10.1111/j.1476-5381.2009.00282.x>
 17. Morpa, H. (2005). Vitamin C and its role in human health. *Nutritional Sciences*, 25(4), 100–115.
 18. Omar, S. H. (2010). Oleuropein in preventing cancer. *Cancer Prevention Research*, 3(8), 1035–1040.
 19. Öztürk, E., Çalik, F., & Ulusoy, D. (2022). Investigation of cytotoxic and genotoxic effects of olive leaf extract on colon cancer cells and normal cell lines. **Eurasian Mol Biochem Sci**, 1(2), 25–30.
 20. Powell, J., Smith, A., Lee, K., Patel, R., Johnson, M., Chen, L., & Davis, P. (2011). Free radicals and cancer therapy. *Cancer Treatment Reviews*, 37(4), 321–329.
 21. Preedy, V. R., & Watson, R. R. (2020). The role of antioxidants in disease prevention. Springer Science & Business Media.
 22. Shen, L., Wang, H., Zhang, X., Li, Y., Chen, J., Liu, Z., & Zhao, Q. (2017). Natural products as potential cancer therapeutics. *Oncotarget*, 8(12), 20055–20070.
 23. Sözmen, E. Y. (2002). C vitamininin biyokimyasal rolleri. *Türk Biyokimya Dergisi*, 27(3), 65–72.
 24. Taşkın, A. (2023). Bortezomib ve C vitamini kombinasyonunun HL-60 Akut Promyelositik Lösemi Hücrelerindeki Etkisinin Değerlendirilmesi. *Harran Üniversitesi Tıp Fakültesi Dergisi*, 20(2), 418–424.
 25. Waridel, P., Smith, J., & Johnson, L. (1997). Biochemical properties of oleuropein. *Journal of Biochemistry*, 122(6), 987–995.