



Assessing of antioxidant activities of, L-carnitine, Coenzyme Q10 and Beta Carotene dietary supplements

Yeşim Özkan Dağlıoğlu^{1*} 

ABSTRACT

In this study, common tests such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, hydrogen peroxide (H₂O₂) and superoxide anion (O₂⁻) radical scavenging activity were exerted to measure the antioxidant activities of L-carnitine, Coenzyme Q10 (Co-Q10) and β-carotene dietary supplements. Antiradical and antioxidant capacities were evaluated comparatively with these tests. The highest free radical scavenging activity was observed in β-carotene (H₂O₂ 73%; O₂⁻ 69%), while the lowest activity was recorded in L-carnitine (H₂O₂ 59%; O₂⁻ 67%). The radical scavenging activity of these dietary supplements was discovered to be significantly different from each other (*P* < 0,05). The antiradical activities of these dietary supplements were measured colorimetrically based on their DPPH radical scavenging ability. At the end of the incubation period (30 minutes), the effective concentration (EC₅₀) values of β-carotene, Co-Q10, L-carnitine and methanol solutions were measured as 10.060, 28.990 and 146.682 µg/mL, respectively. These results demonstrate the potent antioxidant and antiradical activities of all three dietary supplements compared to ascorbic acid (standard). It can only be said that the antiradical activity of L-carnitine is lower than the others, suggesting that this may be due to kinetic behavior.

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Introduction

Free radicals can occur through the environment (external sources) or through natural physiological processes. It can be produced externally as a result of stress, diet, smoking, inflammation, ozone, exercise, drugs, alcohol air pollutants, industrial chemicals, and exposure to X-rays and sunlight. Free radicals, of which there are many types. However, the most common in aerobic (oxygen breathing) organisms are Reactive Oxygen Species (ROS), which are oxygen free radicals. Examples include hydroxyl, superoxide anion, singlet oxygen and hydrogen peroxide. Superoxide radical is taken into account of a primary ROS because it is generated in mitochondria by electron escape from the ETS (electron transport chain) to molecular oxygen in oxidative phosphorylation [1]. The addition of an electron to dioxygen forms the superoxide anion radical (O₂⁻). Superoxide, usually known radical in biological systems, is produced largely in the mitochondria organelle of the cell. Mitochondria perform the task of energy production and cellular respiration through the "electron transport chain" mechanism. Electrons produce chemical energy by transferring from one molecule to another in the ETS. Oxygen, which is ranked last in the ETS. Some of the electrons leaking from the ETS react with oxygen to compose superoxide radicals [2-3]. Approximately 3% of the oxygen in the mitochondria is transformed to superoxide [4-5]. Mitochondrial DNA (mtDNA) is the basic place of radical oxygen injury from superoxide. Although cells can repair most nuclear DNA damage, they cannot easily repair mtDNA damage, and over time mtDNA injury gathers in cells and closes mitochondria. This causes cells to die and the organism to age [1]. However, superoxide radical is also generated by phagocytes to kill pathogens during infection. Superoxide radicals can be produced by both enzymatic and non-enzymatic pathways. Superoxide radicals, which are radicals, are relatively unreactive with biological molecules such as lipids, proteins and DNA. Under physiological situation, the superoxide radical usually exists largely as the radical anion (O₂⁻) and in a very small proportion (0.6%) as the hydroperoxyl radical (HOO·) [6]. Superoxide radicals do not interact straight with DNA Radical anion can be occurred in biological systems as a result of thiol mediated mending of radicals by thiols, outcomes in the occurring of thiyl radicals [7]. Hydrogen peroxide (H₂O₂) is a non-free radical, oxidizing agent generated by the two electron reduction of oxygen (O₂). Hydrogen

¹ OrduUniversity, Faculty of Engineering, Department of Food Engineering, Corum/ Turkey

*Corresponding Author: Yeşim Özkan Dağlıoğlu e-mail: yozkam52@gmail.com

peroxide can produce OH (hydroxyl) through the fenton reaction in existence molecular oxygen (O₂) and transition metal ions. Superoxide and hydrogen peroxide are the two main ROS, which form other radicals such as the hydroxyl radical and peroxynitrite. DPPH is a stable free radical discovered by Blois method [8]. In the DPPH method, the antioxidant capacity is evaluated by measuring the DPPH radical scavenging capacity of antioxidants spectrophotometrically. Supplements are widely used today to protect against various diseases, to strengthen the immune system and for supportive and complementary treatment. The most notable of these supplements are Coenzyme Q10 (Co-Q10), β-carotene and L-carnitine. Each of these has its own specific purpose. Co-Q10, also known as ubiquinone, is a lipid-soluble (lipophilic) intracellular antioxidant that delays aging, improves human immunity, increases body resistance and vitality, reduces oxidative stress in coronary artery disease, and increases antioxidant enzyme activity [9-10]. Co-Q10 is synthesized internally in the human and acting an important role in the formation of cellular respiration in the mitochondria for the production of ATP (adenosine triphosphate). It protects proteins (mitochondrial), cholesterol (low-density lipoprotein) and phospholipids (membrane) from oxidative cell damage caused by free radicals [11]. Co-Q10 behaves as an intracellular antioxidant by behaving as the main quenching of free radicals and reactive oxygen species. β-carotene is one of more than 600 naturally occurring compounds called carotenoids. Carotenoids are pigments that assist in photosynthesis in plants. The antioxidant effect of β-carotene is based on its capability to quenching peroxy radicals and quench singlet oxygen [12-13]. It protects lipids from free radical oxidation by binding to aggressive peroxy free radicals. They can terminate oxidation chain reactions [14-15]. L-carnitine has significant physiological duty ATP manufacturing in peripheral tissues and in carrying long chain fatty acids over the internal mitochondrial membrane for β-oxidation [16-17]. L-carnitine, obtained from both dietary and internally biosynthesis, is an significant co-factor of peroxisomal oxidation of long-chain fatty acids and has significant duties in the regulation and intermediary metabolism of neural functions in humans [18]. The objective of this study is to comparatively appraise the antioxidant capacities of antioxidant supplements such as Co-Q10, l-carnitine and β-carotene, which are used by consumers to protect their general health and support their immunity.

Material and Methods

Material

Ministry of Agriculture and Forestry approved L-carnitine (500 mg per tablet), Coenzyme Q10 (30 mg per tablet), Beta Caroten capsules (7 mg per tablet) were commercially obtained from Solgar .

Sample Preparation

L-carnitine, Co-Q10 (ubiquinone) and β-carotene tablets were homogenized by mortar and pestle. Each 100 mg of homogenized sample was dissolved by incubating in methanol (1:100 ml ratio) for DPPH analysis and in ultrapure water (UPW) for superoxide anion radical and hydrogen peroxide scavenging activity for 24 hours in a dark medium. At the end of this period, these specimens were centrifuged (1800 rpm, 20 min) and the supernatant was collected for analysis.

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity

Free radical scavenging activities of Co-Q10, L-carnitine and β-carotene were measured against DPPH (2,2-diphenyl-1-picrylhydrazyl) [19]. DPPH is a stable free radical that absorbs at 517 nm, and its absorption decreases when reduced with antioxidants. Briefly, 2 mL of methanol solutions of supplements prepared at different concentrations (5 μg/mL, 10 μg/mL, 20 μg/mL, 40 μg/mL, 80 μg/mL, 160 μg/mL, 320 μg/mL, 640 μg/mL and 1280 μg/mL) were combined with DPPH (3 mL) also prepared in methanol. For the reaction, the solutions were incubated in the dark for 30 minutes. Then, absorbance values of the solutions were taken at 517 nm using a spectrophotometer against the DPPH only solution (blank solution). % inhibition activity was calculated according to the equation $[(\%)]=[(A_{\text{control}}-A_{\text{sample}}/A_{\text{control}})\times 100]$. Then, % inhibition was drawn against concentration and EC₅₀ was reckoned from this graph. EC₅₀ values express the concentration at which 50% of radicals are scavenged by antioxidants, with lower EC₅₀ values indicating higher antioxidant capacity. In this study, L-ascorbic acid was employed as the standard antioxidant.

Hydrogen peroxide scavenging activity

H₂O₂ scavenging capability of Co-Q10, L-carnitine and β-carotene was identified by the method of Ruch et al. [20]. For this purpose, 40 mM H₂O₂ solution was made in pH 7.4 PBS (phosphate buffer). 0.6 mL of H₂O₂ was added to Co-Q10, L-carnitine and β-carotene at a concentration of 80 μg/mL. The spectrophotometer absorbance of the reaction mixture was taken at 230 nm after 10 min. PBS was employed as a blank and L-ascorbic acid was employed as a standard. The H₂O₂ clearance percentage of supplements and the standard was calculated according to the equation $[(\% \text{ Cleaned } H_2O_2)]=[(A_{\text{control}}-A_{\text{sample}}/A_{\text{control}})\times 100]$.

Superoxide anion radical scavenging activity

O_2^- scavenging activity of Co-Q10, L-carnitine and β -carotene was measured according to the procedure specified by Nishikimi et al. [21]. Superoxide radicals are formed as a result of the oxidation of NADH and the analysis is based on the reduction of nitroblue tetrazolium (NBT). Briefly, superoxide radicals were produced by mixing 1 ml of NBT solution that was prepared with 156 μ M NBT in 100 mM PBS at pH 8.0, 1 ml of NADH solution that was prepared with 468 μ M NADH in 100 mM PBS at pH 8.0, and 0.1 ml of supplements solution. To initiate the reaction, 100 μ l of PMS solution, which was prepared with 60 μ M PMS in 100 mM PBS at pH 8.0, was added to the mixture. These reaction mixture was kept at 24°C for 7 min and the absorbance at 560 nm was read against blank. L-Ascorbic acid was employed as control. The reducing absorbance of the reaction mixture stated rising superoxide anion scavenging activity. Percent inhibition of superoxide anion formation by supplements and standards was calculated according to the equation [(% SOSA)=[(Acontrol–Asample/Acontrol)×100]. Blank, in which NBT + NADH + PMS solution without supplement was used.

Statistical Analysis

The results obtained were given as mean. Significant differences were defined by one-way analysis of variance (ANOVA) employing the SPSS 26.0 statistical package program. Differences were accepted as significant at the 0.05 level ($P < 0.05$). All experiments were repeated at least three times.

Results and Discussion

Scanning electron analysis results

The O_2^- (superoxide anion radical) is a poor oxidant. However, it is quite toxic (dangerous) and causes oxidative stress by causing the manufacture of singlet oxygen and hydroxyl radicals, which are stronger [22]. Enzymatic reactions in the xanthine oxidase system can produce superoxide anion radical. Hydrogen peroxide (H_2O_2) radical can be produced in organisms by reactions catalyzed by superoxide dismutase (SOD) and many oxidizing enzymes (glucose oxidase, xanthine oxidase and other oxidases). Since the H_2O_2 formed is uncharged, it can pass through biological membranes and oxidize many biomolecules. However, this oxidation is very slow because the reaction kinetics are slow, which makes it possible for them to accumulate in large quantities [23]. The radical quenching abilities of antioxidant compounds are generally related to their potential to generate stable radicals. In this study, the ability of Co-Q10, L-carnitine and β -carotene to scavenge hydrogen peroxide and superoxide anion is demonstrated in Figure 1. The ability of these supplements to scavenge H_2O_2 and O_2^- was measured in comparison to the standard antioxidant ascorbic acid. At the same concentrations (80 μ g/mL), the O_2^- and H_2O_2 scavenging activities (%) of L-carnitine, Co-Q10, and β -carotene and ascorbic acid were 67, 69, 69, 81 and 59, 71, 73, 84, respectively. These results showed that these three supplements have effective superoxide anion radical and hydrogen peroxide scavenging activity and have statistically significant different scavenging effects from each other ($P < 0.05$). At concentrations of 80 μ g/mL, the O_2^- scavenging effect of the supplements and the standard declined in the order of ascorbic acid > Co-Q10 and β -carotene > L-carnitine. H_2O_2 scavenging effect declined in the order of ascorbic acid > β -carotene > Co-Q10 > L-carnitine.

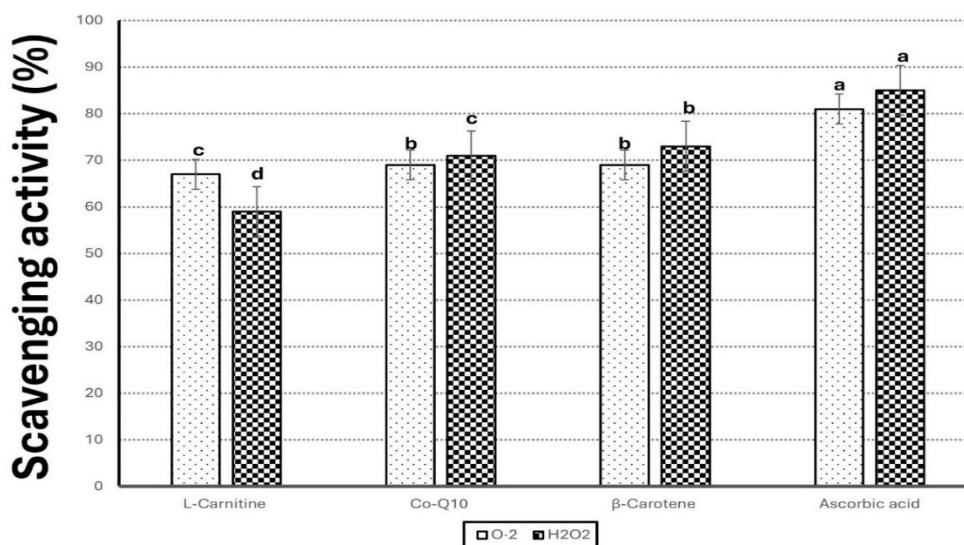


Fig 1 Comparison of O₂⁻ and H₂O₂ radical scavenging activity of Co-Q10, L-carnitine, β-carotene and ascorbic acid at 80 μg/mL concentration. Lower case letters (a,b and c) indicated that the concentrations for each supplements are significantly different from each other according to Tukey's HSD test (*P* < 0.05).

Again, the antioxidant activities of supplements such as L-carnitine, Co-Q10, and β-carotene in the DPPH test with different concentrations of standard antioxidant (ascorbic acid) and regression EC₅₀ values were calculated. The results of the DPPH analysis are expressed as EC₅₀, the value corresponding to the antioxidant concentration necessary to drop the early concentration of the DPPH radical by 50% [24].

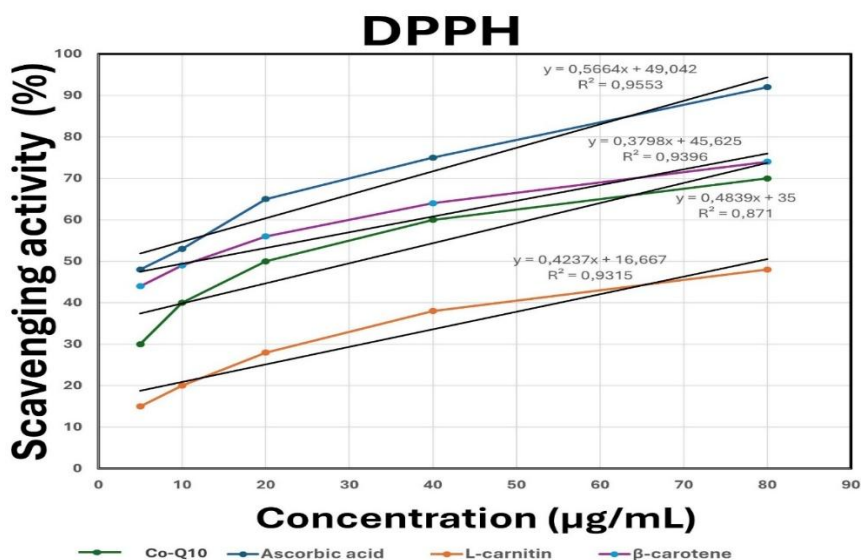


Fig 2 Relationship between Co-Q10, Ascorbic acid, L-carnitine and β-carotene supplement at increasing concentrations and DPPH free radical scavenging activity

In Figure 2, the antioxidant activities of the supplements at changing concentrations (5, 10, 20, 40 and 80 μg/ml) were tested using the DPPH method. In the DPPH assay, antioxidants (i.e., molecules that can donate electrons or hydrogen) colorimetrically drop the purple stable radical DPPH to the yellowish DPPH2 (diphenyl-picrylhydrazine). DPPH gives strong absorption at 517 nm due to unpaired electron. When the radical pairs its unpaired electron in the presence of antioxidant (hydrogen donor), its absorption decreases and its purple color turns into yellow. In other words, the lower the absorption, the higher the antioxidant activity. In our study, it was witnessed that the free radical scavenging activity of Co-Q10, L-carnitine and β-carotene antioxidants changed depending on the concentration change. When the DPPH radical scavenging efficiency of the supplements was tested, β-carotene, Co-Q10 and L-carnitine showed DPPH free radical scavenging efficiency of 74%, 70% and 48% at 80 μg/ml.

Table 1 Co-Q10, L-carnitine, β-carotene and ascorbic acid EC₅₀ values

Samples	EC ₅₀
Ascorbic acid	5.424 μg/mL
β-carotene	10.060 μg/mL
Co-Q10	28.990 μg/mL
L-carnitine	146.682 μg/mL

In the present study, the antiradical activity effective concentrations (EC₅₀) of Co-Q10, L-carnitine and β-carotene were calculated and expressed in Table 1. The EC₅₀ value is a very useful measurement parameter for evaluating antioxidant potential [25]. However, the non-linear relationship between antiradical activity and antioxidant concentration in the calculation of EC₅₀ values makes the EC₅₀ calculation somewhat difficult. The R² values in our study being between 0.9553 that is, very close to 1, indicate that the calibration ranges are

quite linear. In general, the lower the EC₅₀ value, the less antioxidant is needed to scavenge DPPH radicals, indicating the radical scavenging effect of the antioxidant. Of these supplements, β-carotene and Co-Q10 appear to have relatively high antiradical activity compared to L-carnitine. In addition, although the R² (0.9315) value of L-carnitine is high, the linear link between its the percentage of inhibition and concentration is limited. This is clearly seen when we look at Figure 2. For L-carnitine, the Max. concentration (80 μg/ml) needs to be increased to obtain a better result and a non-linear relationship is likely to occur with increasing concentration. A similar situation has been observed in previous studies. For example, in one study, researchers reported that the maximum concentration should demonstrate 670 percent radical scavenging efficiency for the results to be more satisfactory [26]. In a different study, a linear relationship was demonstrated to link antioxidant concentration and percent inhibition in a limited concentration range [27].

DPPH is on a large scale employed to estimate the free radical scavenging efficiency of diverse antioxidant substances. Some of these studies are as follows; To evaluate the O₂⁻ radical scavenging activity of *Vaccinium myrtillus* L. (blueberry), natural antioxidants vitamin C, phenolic acids and flavonoids found in *V. myrtillus* were separated by solid phase extraction. These antioxidants have been shown to have very strong free radical quenching capacities as a result of the superoxide anion free radical test [28]. In the study investigating the role of Coenzyme Q10 in improving the harmful effects of oxidative stress caused by high doses of H₂O₂, it was noted that the oxidative stress effect caused by H₂O₂ could be reduced by the application of Coenzyme Q10 [29]. The antioxidant properties of L-carnitine was researched *in vitro* using the DPPH test, H₂O₂ and O₂⁻ scavenging activities. As a result, it was found that L-carnitine has strong H₂O₂ and O₂⁻ scavenging activities. Its EC₅₀ for DPPH is very high compared to ascorbic acid. Therefore, it cannot be said that L-carnitine has strong DPPH scavenging activity. [30]. In the study researching the link between plasma L-carnitine amount and antioxidant effects, it was noted that L-carnitine increased the capacities of total antioxidant and antioxidant enzymes capacity in healthy individuals. As a result, researchers have suggested that it can be used as a complementary treatment in chronic diseases that cause oxidative stress [31]. In another study, L-carnitine and its effects on antioxidant status and lipid peroxidation were examined in the liver, kidneys, and blood of aged and young rats. L-carnitine has been shown to improve over time the excessively elevated lipid peroxides and excessively decreased antioxidants in elderly individuals [32]. Researchers have highlighted that carnitine is highly effective in normalizing age-related antioxidant status and lipid peroxidation and in regressing age-related disorders of free radicals [32]. In a study evaluating the potential protective effect of L-carnitine, it was shown to be effective in reducing some biochemical and hematological changes and chromosomal damage induced by free radicals caused by radiation in mice [33].

Conclusion

According to the results of the study, commercially available Co-Q10, L-carnitine and β-carotene supplements were found to be effective antioxidants in decreasing power, DPPH radical and H₂O₂ and O₂⁻ scavenging activity assays when encounter with standard antioxidant compounds such as ascorbic acid.

Abbreviations

DDPH: 2,2-diphenyl-1-picrylhydrazyl; ETS: electron transport chain; O₂⁻: Superoxide Anion Radical Canadian; ROS: Reactive Oxygen Species; Co-Q10: Coenzyme Q10; mtDNA: Mitochondrial DNA; HOO·: Hydroperoxyl Radical; H₂O₂: Hydrogen Peroxide; SOD: Superoxide Dismutase; ANOVA: one-way analysis of variance; UPW : Ultrapure Water; PBS: Phosphate Buffer; SD: Standard Deviation

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Availability of data and material

Please contact the corresponding author for any data request.

References

1. Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J., & Telser, J. (2004). Role of oxygen radicals in DNA damage and cancer incidence. *Molecular and cellular biochemistry*, 266, 37-56.
2. Hanukoglu, I., Rapoport, R., Weiner, L., & Sklan, D. (1993). Electron leakage from the mitochondrial NADPH-adrenodoxin reductase-adrenodoxin-P450_{scc} (cholesterol side chain cleavage) system. *Archives of biochemistry and biophysics*, 305(2), 489-498.

3. Salvador, A., Sousa, J., & Pinto, R. E. (2001). Hydroperoxyl, superoxide and pH gradients in the mitochondrial matrix: a theoretical assessment. *Free Radical Biology and Medicine*, 31(10), 1208-1215.
4. Benzi, G. I. A. N., Pastorin, O., Marzatico, F., Villa, R. F., Dagani, F., & Curti, D. (1992). The mitochondrial electron transfer alteration as a factor involved in the brain aging. *Neurobiology of Aging*, 13(3), 361-368.
5. Brookes, P. S., Levenon, A. L., Shiva, S., Sarti, P., & Darley-Usmar, V. M. (2002). Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radical Biology and Medicine*, 33(6), 755-764
6. de Grey, A. D. (2002). HO [sub 2]•: The Forgotten Radical. *DNA & Cell Biology*, 21(4).
7. Valko, M., Rhodes, C. J. B., Moncol, J., Izakovic, M. M., & Mazur, M. (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-biological interactions*, 160(1), 1-40. <https://doi.org/10.1016/j.cbi.2005.12.009>
8. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181(4617), 1199-1200.
9. Ernster, L., & Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, 1271(1), 195-204.
10. Bhagavan, H. N., & Chopra, R. K. (2006). Coenzyme Q10: absorption, tissue uptake, metabolism and pharmacokinetics. *Free radical research*, 40(5), 445-453. <https://doi.org/10.1080/10715760600617843>
11. Singh, U., Devaraj, S., & Jialal, I. (2007). Coenzyme Q10 supplementation and heart failure. *Nutrition Reviews*, 65(6), 286-293.
12. Burton, G. W., & Ingold, K. (1984). β-Carotene: an unusual type of lipid antioxidant. *Science*, 224(4649), 569-573.
13. Stahl, W., & Sies, H. (1996). Lycopene: a biologically important carotenoid for humans?. *Archives of biochemistry and biophysics*, 336(1), 1-9.
14. Britton, G. (1995). Structure and properties of carotenoids in relation to function. *The FASEB Journal*, 9(15), 1551-1558.
15. Rice-Evans, C. A., Sampson, J., Bramley, P. M., & Holloway, D. E. (1997). Why do we expect carotenoids to be antioxidants in vivo?. *Free radical research*, 26(4), 381-398.
16. Shug, A. L., Schmidt, M. J., Golden, G. T., & Fariello, R. G. (1982). The distribution and role of carnitine in the mammalian brain. *Life sciences*, 31(25), 2869-2874.
17. Mroczkowska, J. E., Galla, H. J., Nałecz, M. J., & Nałecz, K. A. (1997). Evidence for an Asymmetrical Uptake of L-Carnitine in the Blood-Brain Barrier in Vitro. *Biochemical and biophysical research communications*, 241(1), 127-131.
18. Ramsay, R. R. (1999). The role of the carnitine system in peroxisomal fatty acid oxidation. *The American journal of the medical sciences*, 318(1), 28-35.
19. Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, 28(1), 25-30
20. Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003-1008.
21. Nishikimi, M., Rao, N. A., & Yagi, K. (1972). The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochemical and biophysical research communications*, 46(2), 849-854.
22. Liu, X., Zhao, M., Wang, J., Yang, B., & Jiang, Y. (2008). Antioxidant activity of methanolic extract of emblica fruit (*Phyllanthus emblica* L.) from six regions in China. *Journal of food composition and Analysis*, 21(3), 219-228. <https://doi.org/10.1016/j.jfca.2007.10.001>
23. Andrés, C. M. C., Pérez de la Lastra, J. M., Juan, C. A., Plou, F. J., & Pérez-Lebeña, E. (2022). Chemistry of hydrogen peroxide formation and elimination in mammalian cells, and its role in various pathologies. *Stresses*, 2(3), 256-274. <https://doi.org/10.3390/stresses2030019>
24. Sridhar, K., & Charles, A. L. (2019). In vitro antioxidant activity of Kyoho grape extracts in DPPH and ABTS assays: Estimation methods for EC50 using advanced statistical programs. *Food Chemistry*, 275, 41-49. <https://doi.org/10.1016/j.foodchem.2018.09.040>
25. Locatelli, M., Gindro, R., Travaglia, F., Coisson, J. D., Rinaldi, M., & Arlorio, M. (2009). Study of the DPPH-scavenging activity: Development of a free software for the correct interpretation of data. *Food chemistry*, 114(3), 889-897. <https://doi.org/10.1016/j.foodchem.2008.10.035>
26. Buenger, J., Ackermann, H., Jentzsch, A., Mehling, A., Pfitzner, I., Reiffen, K. A., ... & Wollenweber, U. (2006). An interlaboratory comparison of methods used to assess antioxidant potentials I. *International Journal of Cosmetic Science*, 28(2), 135-146. <https://doi.org/10.1111/j.1467-2494.2006.00311.x>
27. Villaño, D., Fernández-Pachón, M. S., Troncoso, A. M., & García-Parrilla, M. C. (2005). Comparison of antioxidant activity of wine phenolic compounds and metabolites in vitro. *Analytica Chimica Acta*, 538(1-2), 391-398. <https://doi.org/10.1016/j.aca.2005.02.016>
28. Tumbas, V., Čanadanović-Brunet, J., Gille, L., Dilas, S., & Četković, G. (2010). Superoxide anion radical scavenging activity of bilberry (*Vaccinium myrtillus* L.). *Journal of Berry Research*, 1(1), 13-23. <https://doi.org/10.3233/BR-2010-002>
29. Humaish, H. H. (2015). Hepatoprotective effect of Co-enzyme Q10 in rats treated with Hydrogen Peroxide. *Journal of Wasit for Science and Medicine*, 8(2), 52-64.
30. Gülçin, I. (2006). Antioxidant and antiradical activities of L-carnitine. *Life sciences*, 78(8), 803-811. <https://doi.org/10.1016/j.lfs.2005.05.103>
31. Cao, Y., Qu, H. J., Li, P., Wang, C. B., Wang, L. X., & Han, Z. W. (2011). Single dose administration of L-carnitine improves antioxidant activities in healthy subjects. *The Tohoku journal of experimental medicine*, 224(3), 209-213. <https://doi.org/10.1620/tjem.224.209>
32. Kalaiselvi, T., & Panneerselvam, C. (1998). Effect of L-carnitine on the status of lipid peroxidation and antioxidants in aging rats. *The Journal of Nutritional Biochemistry*, 9(10), 575-581. [https://doi.org/10.1016/S0955-2863\(98\)00052-7](https://doi.org/10.1016/S0955-2863(98)00052-7)
33. Mohamed, N. E., & Farghaly, A. A. (2009). Evaluation of the protective effect of L-carnitine on radiation induced free oxygen radicals and genotoxicity in male mice. *Researcher*, 1(6), 7-15.