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# Spectrophotometric Evaluation of Total Antioxidant Capacity Using the Phosphomolybdenum Complex Formation Method

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***Abstract***— *The present study evaluated the antioxidant capacity of selected plant extracts using the phosphomolybdenum assay, a spectrophotometric method based on the reduction of molybdenum (VI) to molybdenum (V) and the subsequent formation of a green phosphate/Mo(V) complex under acidic conditions. The absorbance of the resulting complex was measured at 695 nm and compared with the synthetic antioxidant butylated hydroxytoluene (BHT) as the reference standard. Relative Antioxidant Capacity (rAOC) was determined using the ratio of the slope of each sample to the slope of BHT.*

*Results showed a concentration-dependent increase in absorbance for all samples, indicating their ability to reduce molybdenum ions and exhibit antioxidant activity. The calculated rAOC values from the seven-point calibration were 0.39, 0.55, and 0.45 for CcC, SiC, and DcH, respectively, while the three-point calibration yielded rAOC values of 0.42, 0.55, and 0.45. BHT consistently exhibited the highest antioxidant capacity (rAOC = 1.00). Among the plant extracts, SiC demonstrated the greatest antioxidant activity, followed by DcH and CcC. However, comparison of the regression slopes and rAOC values revealed no significant differences among the samples. The graphical analysis further confirmed the similarity of the antioxidant responses, with only a slight variation observed in the rAOC value of CcC between the three-point and seven-point analyses.*

*These findings suggest that all tested extracts possess measurable antioxidant potential, although their activities remain lower than that of BHT. The absence of significant differences among the extracts indicates comparable antioxidant capacities under the conditions of the phosphomolybdenum assay. The observed antioxidant properties support the potential use of these natural plant sources in developing prophylactic and therapeutic agents against oxidative stress-related diseases, including cancer and cardiovascular disorders. Furthermore, the results provide scientific support for the therapeutic claims associated with antioxidant-rich natural herbs and warrant further investigation into their bioactive constituents and pharmacological applications.*

***Keywords***— *Antioxidant capacity, phosphomolybdenum assays, UV-Vis spectrophotometry, BHT, plant extracts, rAOC.*

## I. Introduction

Antioxidants have gained considerable attention in food, pharmaceutical, and cosmetic research because of their ability to counteract oxidative damage and promote human health [1]. Oxidative stress occurs when the production of reactive oxygen species exceeds the capacity of endogenous defense mechanisms, leading to cellular and molecular damage that contributes to aging and the development of chronic diseases [2]. Extensive evidence has linked oxidative stress to the pathogenesis of cardiovascular diseases, cancer, diabetes mellitus, neurodegenerative disorders, and inflammatory conditions, highlighting the importance of identifying and evaluating effective antioxidant sources [3][4][5]. Natural plant-derived antioxidants have become particularly attractive due to their abundance of bioactive compounds, including phenolics, flavonoids, tannins, and other secondary metabolites that can scavenge free radicals and inhibit oxidative reactions [6].

The assessment of antioxidant capacity is therefore essential for determining the potential health-promoting properties of plant-derived extracts and functional products [7]. Several analytical methods have been developed to evaluate antioxidant activity; however, differences in reaction mechanisms and antioxidant behavior often result in varying estimates of antioxidant potential [8]. Among these methods, the phosphomolybdenum assay introduced by Prieto et al. remains one of the most widely accepted techniques for its simplicity, reproducibility, sensitivity, and applicability to both water- and lipid-soluble antioxidant compounds [9]. The assay is based on the reduction of molybdenum (VI) to molybdenum (V) by antioxidant substances under acidic conditions, resulting in the formation of a green phosphate–Mo(V) complex that can be quantified spectrophotometrically [10],[11].

Given the increasing interest in natural antioxidant sources and the need for efficient analytical approaches, this study aims to determine and compare the total antioxidant capacities of selected plant extracts using the phosphomolybdenum method. Furthermore, the study evaluates the efficiency of reduced-calibration-point measurements as a potential strategy to simplify antioxidant analysis while maintaining analytical reliability and accuracy [12].

## II. Materials and Methods

*Chemicals and Reagents*. The antioxidant capacity of the plant extracts CcC, SiC, and DcH was determined using the phosphomolybdenum assay. The chemicals and reagents used in the study included methanol, concentrated sulfuric acid ($H_2SO_4$), sodium phosphate ($Na_3PO_4$), ammonium molybdate tetrahydrate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$], and butylated hydroxytoluene (BHT), which served as the reference antioxidant standard. All reagents were of analytical grade and were used without further purification.

*Instrumentation.* The instruments and laboratory apparatus used in this study included a Double Beam UV–Visible Spectrophotometer (LI-2800, LI Sciences), 50-mL volumetric flask, automatic micropipettes (0.3 mL and 3.0 mL), disposable UV cuvettes (2.5 mL), capped test tubes, capped sample vials,



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a thermometer, a hot plate, and a boiling water bath. Absorbance measurements were performed at a wavelength of 695 nm using the UV–Visible spectrophotometer.

*Sample Preparation.* The phosphomolybdenum reagent solution consisted of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The reagent was prepared by dissolving 0.2295 g of sodium phosphate and 0.2472 g of ammonium molybdate in a methanolic sulfuric acid solution containing 1.6 mL of concentrated sulfuric acid, then adjusting the final volume to 50 mL with methanol.

Sample solutions were prepared by dissolving 0.0500 g of each dried plant extract (CcC, SiC, and DcH) in 1.0 mL methanol. A stock solution of BHT (150 mg/mL) was prepared in methanol and subsequently diluted to obtain standard concentrations for the calibration curve.

*Evaluation of total antioxidant capacity.* The total antioxidant capacity of the plant extracts was determined following the phosphomolybdenum method described by Prieto et al. Briefly, 3.0 mL of the phosphomolybdenum reagent solution was transferred into each test tube. Subsequently, 0.3 mL of either plant extract solution, BHT standard solution, or methanol (blank control) was added. Sample analyses were conducted in triplicate, while standard solutions were prepared in single determinations.

The reaction mixtures were capped and incubated in a boiling water bath maintained at 95°C for 90 min. After incubation, the tubes were allowed to cool to room temperature for approximately 50 min. The absorbance of the resulting green phosphate/Mo(V) complex was measured at 695 nm against the methanol blank.

For calibration, a series of BHT standard solutions was prepared by transferring appropriate aliquots (100, 200, 400, 600, 800, 1000, and 1300 µL) of the stock solution into separate vials and adjusting the volume to 5 mL with methanol. Calibration curves were generated by plotting absorbance against BHT concentration. The antioxidant capacities of CcC, SiC, and DcH were expressed relative to the BHT standard.

The relative antioxidant capacity (rAOC) of each sample was calculated using the equation:

$$\text{rAOC} = (\text{Slope of Sample}) / (\text{Slope of BHT})$$

where the slope was obtained from the linear regression equation of the concentration–absorbance calibration plot. Results were reported as mean ± standard deviation of triplicate determinations.

## III. Results and Discussion

The antioxidant capacities of the plant extracts CcC, SiC, and DcH were evaluated using the phosphomolybdenum assay and expressed as relative antioxidant capacity (rAOC) in comparison with the reference antioxidant, butylated hydroxytoluene (BHT). The assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds, resulting in the formation of a green phosphate/Mo(V) complex that is measured spectrophotometrically at 695 nm.

The concentration–absorbance plots for all samples exhibited a positive linear relationship, indicating that antioxidant activity increased proportionally with increasing sample concentration. Similar trends were observed for both the seven-point and three-point calibration models. The linear regression slopes obtained from the concentration–absorbance plots were used to calculate the relative antioxidant capacity of each sample.

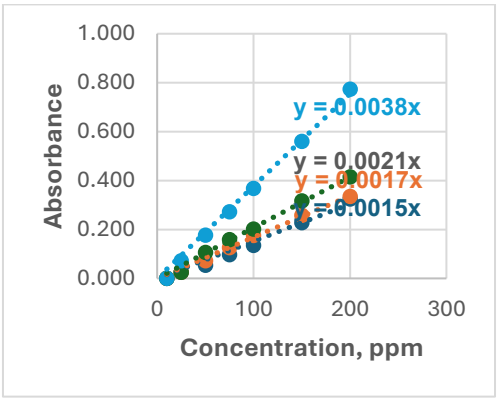


Figure 1. Seven-data points plot of all samples and BHT

For the seven-point calibration shown in Figure 1, the calculated relative antioxidant capacity (rAOC) values were 0.39, 0.55, and 0.45 for CcC, SiC, and DcH, respectively, while butylated hydroxytoluene (BHT) exhibited an rAOC value of 1.00. Similarly, the three-point calibration presented in Figure 2 yielded rAOC values of 0.42, 0.55, and 0.45 for CcC, SiC, and DcH, respectively, with BHT again assigned a value of 1.00. The comparable results from both calibration approaches suggest that the reduced calibration model can reliably estimate antioxidant capacity while minimizing analytical complexity and reagent consumption [13]. Among the plant extracts evaluated, SiC exhibited the highest antioxidant capacity, suggesting a greater abundance or greater effectiveness of redox-active phytochemicals that can donate electrons and neutralize reactive species [14].

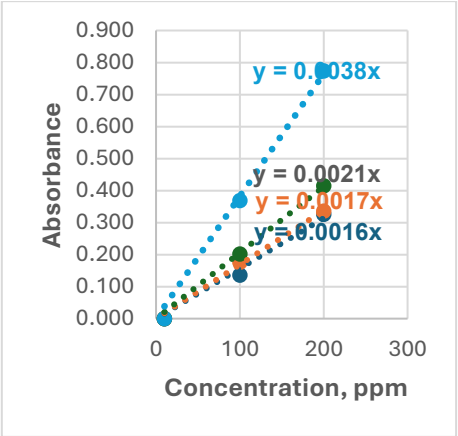


Figure 2. Three-data-point plot of all samples and BHT

Although all plant extracts exhibited lower antioxidant capacities than BHT, their measurable activity confirms the presence of naturally occurring antioxidant compounds that may contribute to the reduction of oxidative stress and biological protection [15]. Previous studies have shown that plant-derived antioxidants, particularly phenolic and flavonoid



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constituents, often exhibit lower in vitro antioxidant values than synthetic standards but are valued for their safety, bioavailability, and multifunctional health-promoting properties [14,15]. These findings support the potential application of the tested extracts as natural antioxidant sources and further demonstrate the suitability of simplified calibration procedures for antioxidant screening assays [13].

Among the tested extracts, SiC demonstrated the highest antioxidant capacity, with an rAOC value of 0.55 in both calibration approaches. This finding suggests that SiC contains a higher concentration of electron-donating antioxidant constituents that facilitate the reduction of molybdenum ions during the phosphomolybdenum reaction, thereby reflecting a stronger total antioxidant potential [16]. DcH exhibited intermediate antioxidant activity with an rAOC value of 0.45, while CcC showed the lowest antioxidant capacity, with rAOC values ranging from 0.39 to 0.42. Variations in antioxidant capacity among plant extracts are often attributed to differences in the composition and concentration of bioactive compounds, particularly phenolics, flavonoids, and other secondary metabolites that contribute to redox activity and free radical scavenging mechanisms [17]. Despite these differences, the relatively narrow range of rAOC values observed among the extracts indicates that all samples possess comparable antioxidant potential under the assay conditions employed. Similar observations have been reported in comparative antioxidant studies, in which extracts with differing phytochemical profiles exhibited moderate variation in antioxidant activity while maintaining comparable overall reducing capacity [18]. These results suggest that while SiC may represent the most promising antioxidant source among the tested extracts, DcH and CcC likewise contain biologically relevant antioxidant compounds that warrant further phytochemical characterization and functional evaluation.

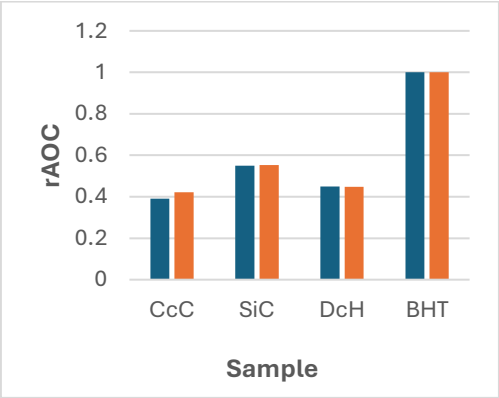


Figure 3. Relative Antioxidant Capacity (rAOC) of the plant samples

The concentration–absorbance plots further support the antioxidant profiles of the tested extracts. The regression slopes obtained from the seven-point calibration were approximately 0.0016, 0.0021, and 0.0017 for CcC, SiC, and DcH, respectively, compared with 0.0041 for BHT, while the three-point calibration produced comparable slopes of approximately 0.0015, 0.0021, and 0.0017 for CcC, SiC, and DcH, respectively, against 0.0038 for BHT. The strong agreement between the slope values generated by the two calibration approaches demonstrates the precision and reproducibility of the phosphomolybdenum assay, indicating that reduced calibration models can provide reliable estimations of antioxidant capacity without substantially affecting analytical performance [19,20]. The slight increase in the rAOC value of CcC from 0.39 to 0.42 between calibration approaches may reflect minor analytical variability commonly observed in spectrophotometric antioxidant assays, whereas the identical rAOC values obtained for SiC and DcH confirm the stability and consistency of their antioxidant responses [21,22].

The lower antioxidant capacities of the plant extracts compared with BHT are expected, as synthetic antioxidants are highly purified compounds specifically designed to exhibit strong reducing and radical-scavenging activities [23]. Nevertheless, the measurable antioxidant capacities observed in CcC, SiC, and DcH suggest the presence of naturally occurring phytochemicals capable of participating in electron-transfer reactions and neutralizing oxidative species [24]. Numerous studies have demonstrated that phenolic acids, flavonoids, tannins, terpenoids, and other plant-derived metabolites contribute significantly to antioxidant activity through hydrogen atom transfer, metal-chelating activity, and free radical scavenging mechanisms [25]. The effectiveness of these compounds is often influenced by their structural characteristics, concentration, and synergistic interactions within the plant matrix, which can collectively enhance overall antioxidant performance [26].

Overall, the phosphomolybdenum assay confirmed that all three plant extracts possess antioxidant activity, with SiC exhibiting the highest relative antioxidant capacity among the samples evaluated. The close correspondence of rAOC values obtained from both calibration approaches provides evidence of the robustness and repeatability of the analytical method, reinforcing its suitability for antioxidant screening studies [27]. Furthermore, the consistent antioxidant responses observed across the extracts support their potential as natural sources of bioactive compounds with protective effects against oxidative stress [28]. These findings provide an important foundation for future phytochemical profiling, compound isolation, and biological investigations aimed at identifying the specific constituents responsible for the observed antioxidant activities and assessing their potential applications in food, pharmaceutical, and nutraceutical formulations [29][30].

## IV. Conclusion

The phosphomolybdenum assay demonstrated that all plant extracts (CcC, SiC, and DcH) possess antioxidant activity, as evidenced by their ability to reduce Mo(VI) to Mo(V). Among the samples, SiC exhibited the highest relative antioxidant capacity (rAOC = 0.55), followed by DcH (rAOC = 0.45) and CcC (rAOC = 0.39–0.42). Although the antioxidant capacities of the extracts were lower than those of the reference standard BHT, the results indicate that the tested plant extracts contain bioactive compounds with antioxidant potential. Furthermore, the comparable rAOC values obtained from both the three-point and seven-point calibration methods confirm the assay's consistency and reliability. These findings support the potential

of CcC, SiC, and DcH as natural sources of antioxidants and provide a basis for further phytochemical and pharmacological investigations.

## REFERENCES


[1] Apak, R., Özyürek, M., Güçlü, K., & Çapanoğlu, E. (2016). Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. *Journal of Agricultural and Food Chemistry, 64*(5), 997–1027. https://doi.org/10.1021/acs.jafc.5b04739

[2] Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging, 13*, 757–772. https://doi.org/10.2147/CIA.S158513

[3] Sies, H., Jones, D. P., & Berndt, C. (2017). Oxidative stress. *Annual Review of Biochemistry, 86*, 715–748. https://doi.org/10.1146/annurev-biochem-061516-045037

[4] Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., & Bitto, A. (2017). Oxidative stress: Harms and benefits for human health. *Oxidative Medicine and Cellular Longevity, 2017*, Article 8416763. https://doi.org/10.1155/2017/8416763

[5] Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., Rajkovic, J., Tsouh Fokou, P. V., Azzini, E., Peluso, I., Prakash Mishra, A., Nigam, M., El Rayess, Y., Beyrouthy, M. E., Polito, L., Iriti, M., Martins, N., & Sharifi-Rad, J. (2020). Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Frontiers in Physiology, 11*, 694. https://doi.org/10.3389/fphys.2020.00694

[6] Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects. *Journal of Functional Foods, 18*, 820–897. https://doi.org/10.1016/j.jff.2015.06.018

[7] Cory, H., Passarelli, S., Szeto, J., Tamez, M., & Mattei, J. (2018). The role of polyphenols in human health and food systems. *Nutrients, 10*(7), 987. https://doi.org/10.3390/nu10070987

[8] Kumar, N., & Goel, N. (2019). Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnology Reports, 24*, e00370. https://doi.org/10.1016/j.btre.2019.e00370

[9] Alam, M. N., Bristi, N. J., & Rafiquzzaman, M. (2013). Review on in vivo and in vitro methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal, 21*(2), 143–152. https://doi.org/10.1016/j.jsps.2012.05.002

[10] Apak, R., Capanoglu, E., & Shahidi, F. (2018). *Measurement of antioxidant activity and capacity: Recent trends and applications*. Wiley-Blackwell.

[11] Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry, 269*(2), 337–341. https://doi.org/10.1006/abio.1999.4019

[12] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis, 19*(6–7), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003

[13] Benzie, I. F. F., & Devaki, M. (2018). The ferric reducing antioxidant power (FRAP) assay for measuring antioxidant capacity. In *Oxidants and Antioxidants Part A* (pp. 77–106). Academic Press. https://doi.org/10.1016/bs.mie.2017.11.004

[14] Tungmunnithum, D., Thongboonyou, A., Pholboon, A., & Yangsabai, A. (2018). Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines, 5*(3), 93. https://doi.org/10.3390/medicines5030093

[15] Salehi, B., Sharifi-Rad, J., Cappellini, F., Reiner, Ž., Zorzan, D., Imran, M., Sener, B., & Sharifi-Rad, M. (2020). The therapeutic potential of anthocyanins: Current approaches based on their molecular mechanism of action. *Frontiers in Pharmacology, 11*, 1300. https://doi.org/10.3389/fphar.2020.01300

[16] Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry, 125*(2), 288–306. https://doi.org/10.1016/j.foodchem.2010.08.012

[17] Dai, J., & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*(10), 7313–7352. https://doi.org/10.3390/molecules15107313

[18] Kasote, D. M., Katyare, S. S., Hegde, M. V., & Bae, H. (2015). Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences, 11*(8), 982–991. https://doi.org/10.7150/ijbs.12096

[19] Gorinstein, S., Leontowicz, H., Leontowicz, M., Namiesnik, J., Najman, K., Drzewiecki, J., Cvikrová, M., Martincová, O., Katrich, E., & Trakhtenberg, S. (2011). Comparison of the main bioactive compounds and antioxidant activities in garlic and white and red onions after treatment protocols. *Journal of Agricultural and Food Chemistry, 59*(9), 4418–4426. https://doi.org/10.1021/jf1042894

[20] Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry, 53*(6), 1841–1856. https://doi.org/10.1021/jf030723c

[21] Floegel, A., Kim, D. O., Chung, S. J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich foods. *Journal of Food Composition and Analysis, 24*(7), 1043–1048. https://doi.org/10.1016/j.jfca.2011.01.008

[22] Cömert, E. D., & Gökmen, V. (2018). Evolution of food antioxidants as a core topic of food science for a century. *Food Research International, 105*, 76–93. https://doi.org/10.1016/j.foodres.2017.10.056

[23] Carocho, M., & Ferreira, I. C. F. R. (2013). A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies. *Food and Chemical Toxicology, 51*, 15–25. https://doi.org/10.1016/j.fct.2012.09.021

[24] Tsao, R. (2010). Chemistry and biochemistry of dietary polyphenols. *Nutrients, 2*(12), 1231–1246. https://doi.org/10.3390/nu2121231

[25] Kumar, S., & Pandey, A. K. (2013). Chemistry and biological activities of flavonoids: An overview. *The Scientific World Journal, 2013*, 162750. https://doi.org/10.1155/2013/162750

[26] Crozier, A., Jaganath, I. B., & Clifford, M. N. (2009). Dietary phenolics: Chemistry, bioavailability and effects on health. *Natural Product Reports, 26*(8), 1001–1043. https://doi.org/10.1039/B802662A

[27] Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*(1), 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

[28] Zielińska, D., Zieliński, H., & Laparra-Llopis, J. M. (2016). Antioxidant capacity and bioactive compounds in selected plant materials. *Food Chemistry, 203*, 404–412. https://doi.org/10.1016/j.foodchem.2016.02.089

[29] Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity in food and biological systems. *Antioxidants & Redox Signaling, 22*(14), 1263–1277. https://doi.org/10.1089/ars.2014.6055

[30] Sharifi-Rad, J., Rodrigues, C. F., Stojanović-Radić, Z., Dimitrijević, M., Aleksić, A., Neffe-Skocińska, K., Zielińska, D., Kołożyn-Krajewska, D., Salehi, B., Prabu, S. M., Mishra, A. P., & Martins, N. (2020). Health-promoting effects of plant-derived antioxidants and their role in disease prevention. *Molecules, 25*(24), 5921. https://doi.org/10.3390/molecules25245921