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Antioxidant Activity of Herbs, Spices, Vegetables, and Dietary Supplements

Alexandra Apostu
Department of Chemistry
University of West Georgia
1601 Maple Street
Carrollton, GA 30118

Faculty Advisor: Dr. Victoria Geisler

Abstract

The field of antioxidant research and their potential health benefits has been getting more attention in recent years. This study looks at a diverse set of food items and contrasts their antioxidant density. A DPPH assay (1,1-diphenyl-2-picrylhydrazyl) was used to determine the antioxidant capacity of herbs, spices, vegetables, and dietary supplements. The antioxidant capacity of each compound was determined through the calculation of IC_{50}. The IC_{50} value specifically refers to the concentration of food extract necessary to reduce the absorbance of the DPPH radical by 50%. The mean IC_{50} values were determined for a variety of samples. Green tea, matcha tea, cinnamon, and cloves had the greatest antioxidant capacity of the material tested.

Keywords: Antioxidants; DPPH Assay; Inhibition Concentration 50%

1. Introduction

This study looks at everyday food items and their overall potency to fight free radicals. Free radicals are either produced by the body via normal metabolic processes or introduced in the body by exposure to the environment. Known examples of free radicals produced by the human body include hydroxyl, peroxyl, and superoxide radicals.[1] On the other hand, environmental factors that could contribute to free radical formation include pollution, medications, alcohol, and tobacco.[2] Excessive production of free radicals can throw off the body's balance between free radicals and antioxidants, leading to oxidative stress.[3] Oxidative stress can damage lipids, proteins, and nucleic acids.[1] As a result, oxidative stress could be responsible for inflammatory diseases, heart diseases, hypertension, Parkinson's disease, and many others.[4]

The body is capable of producing antioxidants such as glutathione, ubiquinol, and uric acid.[3] However, the body cannot produce all the micronutrients it requires through normal metabolic processes. Hence, the primary essential antioxidants such as vitamin E, vitamin C, and beta-carotene must be supplied in the diet.[5]

The United States Dietary Guidelines have steadily been recommending daily consumption of fruits and vegetables due to their high content of vitamins and minerals.[6] In addition to fruits and vegetables, this study has also looked into other rich sources of antioxidants that include herbs, spices, teas, and coffees. Altogether, the purpose behind this research has been to provide the public with a clear picture of nature's most potent resources that prevent and fight off inflammatory diseases.

This study can serve as a foundation for gaining new insight on the mechanism of antioxidants in the body. New information regarding the potency of the studied foods could be derived by investigating the serving size and cooking methods. In addition, future studies can utilize the information presented in this research to determine how the most potent sources of food supplement the treatment of inflammatory diseases, heart diseases, and cancers.

1.1. General Mechanism of Antioxidants

The general mechanism of the reaction between antioxidants and free radicals is by either hydrogen atom transfer (HAT) or single electron transfer (SET).[7] In the SET reaction, Figure 1, the antioxidant will donate an electron to the free radical and will itself become a radical cation. Most often, HAT and SET reactions may occur concurrently.

The concerted movement of a proton and electron is depicted in Figure 2. More specifically, the free radical removes a proton from the antioxidant, which leads to stabilization of the free radical. Once the free radical is stabilized, the antioxidant will take on the role of a free radical.

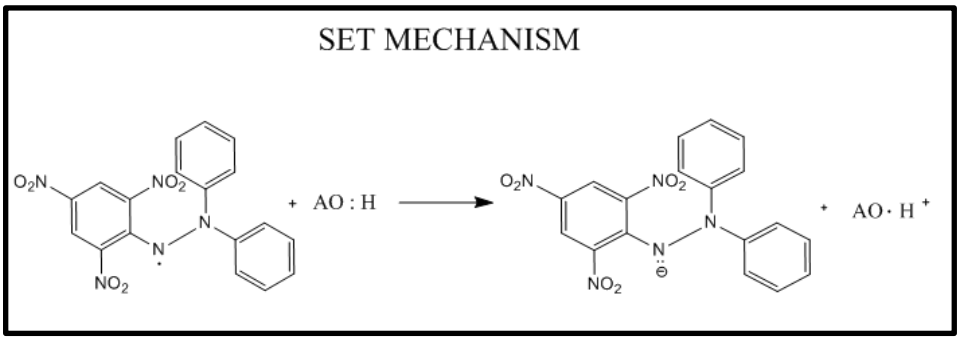

Figure 1. General SET reaction

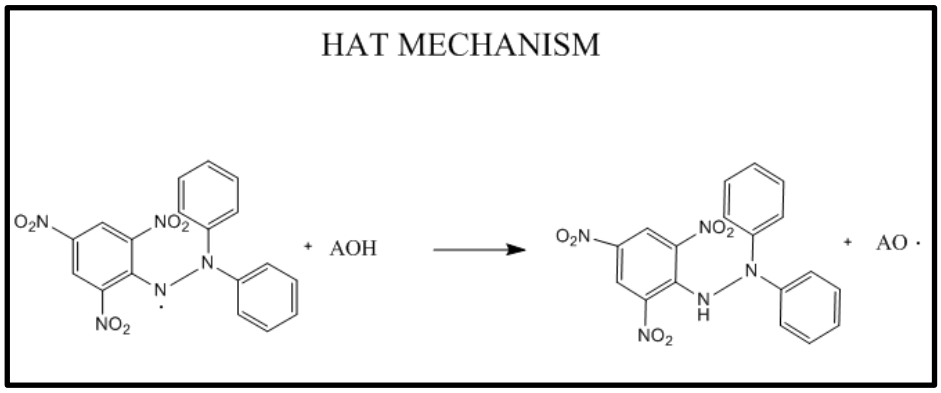

Figure 2. Shows general HAT reaction

The reaction between a free radical and an antioxidant falls under the category of a reduction - oxidation reaction (redox). A redox reaction is a type of chemical reaction that involves the transfer of electrons between two reactants. The species that gains an electron becomes reduced, while the species that loses an electron becomes oxidized. During the reaction examined in this study, the antioxidant transfers an electron to the free radical. As a result, the antioxidant is the species that becomes oxidized. DPPH, the free radical utilized in this study, is the species that becomes reduced by gaining an electron.

2. Methodology

2.1. Preparation of Dehydrated Compounds

Fresh fruits and vegetables are known to have a considerable water content that may cause unwanted dilution of the stock solution. To prevent this, all fresh foods used in this study were dehydrated at 57°C for ~ 24 hours. Once dehydrated, the compounds were ground into a fine powder to facilitate their solubility in 80% ethanol. This procedure was not enforced for compounds that were readily found in a dehydrated, powdered form - such as: dried herbs, teas, and coffee.

2.2. Preparation of DPPH Stock Solution

The DPPH solid was weighed (0.10 g), dissolved and diluted to 200 mL with 80% ethanol. DPPH solution was prepared fresh daily, no longer than 30 minutes prior to being utilized. Once prepared, the 1.3 mM DPPH solution was stored in a covered amber jar.

2.3. Preparation of Antioxidant Extract

A weighted amount (ranging from 0.20 - 7.5 g) of the dehydrated food was added to 10 mL of 80% ethanol and stirred for 25 minutes. The solution was vacuum filtered and washed with an additional 10 mL of 80% ethanol. The obtained filtrate was further diluted to 50 mL using volumetric glassware to give us our stock solution. Using an Eppendorf pipette, 200 µL of the stock solution was diluted to 10 mL using volumetric glassware. This dilution process was repeated with 400 µL, 800 µL, 1600 µL, and 3200 µL of the stock solution. The 10 mL dilutions were stored in amber jars for the remainder of the procedure. In the next step, 250 µL of one of the 10 mL dilutions was placed in a glass disposable culture tube (13x100mm). To the same tube, 2500 µL of the 1.3 mM DPPH was added and the mixture was stirred well. The procedure was repeated for all dilutions and the cuvettes were incubated in the dark for 30 min at room temperature. A Spectronic 20 Genesys spectrophotometer was utilized to measure the absorbance of each tube at 517 nm. The absorbance of the DPPH radical with 250 µL of 80% ethanol, i.e. control, was also measured. All determinations were performed in duplicates.

2.4. Determination of IC_{50}

All absorbance data was collected and the average was calculated for each dilution. The percent inhibition was determined using the following formula, where A_C is the absorption of the control and A_S is the average absorption for each dilution.

$$\text{Percent Inhibition} = (A_C - A_S)/A_C \times 100 \quad (1)$$

The data was plotted using concentration of each food extract (mg/mL) vs the percent inhibition. The calculation of the inhibition concentration at 50% was determined using the slope of the graph, $y = mx + n$. Each compound was re-tested several times to ensure accuracy and an average IC_{50} value was obtained for each compound.

3. Results

The ability of ethanolic food extracts to scavenge free radicals was determined using the DPPH assay. Table 1 shows the IC_{50} values for the drinks, spices and foods tested. The IC_{50} values ranged from 0.21 to 77 mg/mL with teas and spices having the highest levels to parsley, carrots, and ginseng having some of the lowest values.

Table 1. IC_{50} values and standard deviation for all tested compounds

Compound Tested	IC_{50} ± Standard Deviation (mg/mL)	Compound Tested	IC_{50} ± Standard Deviation (mg/mL)
Green Tea	0.21 ± 0.016	Hibiscus Tea	13 ± 1.5
Matcha Green Tea	0.33 ± 0.087	Black Tea	13 ± 1.6
Cinnamon	0.36 ± 0.044	Chrysanthemum Tea	14 ± 1.9
Cloves	0.36 ± 0.061	Dandelion Root	14 ± 4.0
Centrum	0.84 ± 0.24	Collard Greens	15 ± 3.8
Olive Leaves Tea	1.8 ± 0.41	Swiss Chard	17 ± 1.7
Rosemary	2.7 ± 1.2	Black Pepper	17 ± 2.8
Coffee	3.0 ± 1.1	Calendula Tea	18 ± 8.6
Turmeric	3.5 ± 1.3	Red Kidney Beans	21 ± 5.6
Cocoa Powder	4.1 ± 0.90	Romaine	21 ± 6.8
Ginger Root	5.2 ± 0.36	Spinach	29 ± 14
Kale	6.7 ± 3.0	Pu-erh Tea	33 ± 5.8
Thyme	6.7 ± 2.9	Sweet Potatoes	45 ± 9.7
Cumin	7.0 ± 1.5	Parsley	54 ± 8.2
Broccoli	12 ± 4.1	Carrots	57 ± 10.
Jalapeño	13 ± 1.4	Chia Seeds	60 ± 23
		Ginseng	77 ± 23

3.1. Statistical Analysis

Figure 3. shows two comparative examples of the regression lines for a tested food. The orange regression line has a corresponding r^2 value of 0.8756, while the blue regression line has a corresponding r^2 value of 0.9989. To ensure high quality data collection, the concentration of the stock solutions were adjusted in the laboratory to give us the best straight line that would pass through the origin point, with small exceptions. The graphs which displayed an r^2 value lower than 0.7 were excluded from the calculation of average IC_{50}. In addition to the mean IC_{50}, the standard deviation of the mean were also calculated.

Figure 4. shows the color change from purple to light yellow of DPPH with increasing amounts of coffee extract. The concentration of coffee increases towards the right. Consequently, the higher the concentration of coffee extract, the larger the antioxidant activity. As the antioxidant activity increases, more of the DPPH radical is being reduced causing the purple color to change to yellow.

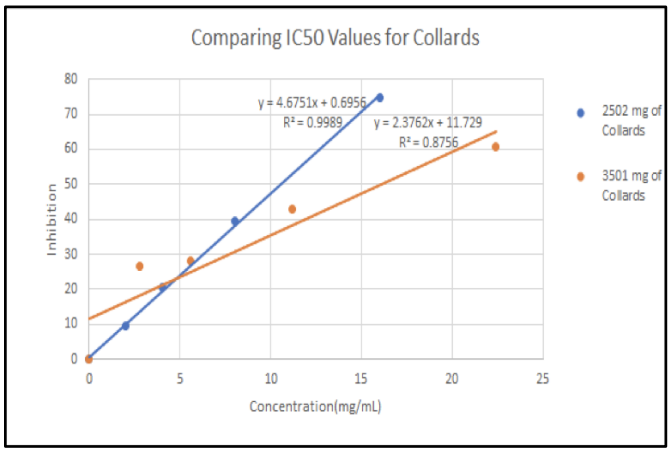

Figure 3. Plot of % inhibition vs. concentration of extract added for the color of DPPH at constant-volume colorimetric titration of DPPH with collard extract.

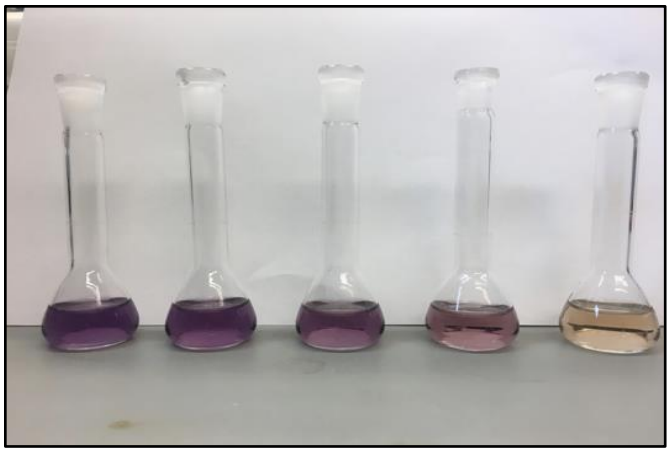

Figure 4. Shows the gradual color change for coffee.

3.2. Antioxidant Capacity Comparisons

When looking at the vegetables, legumes and seeds tested, Figure 5, broccoli had the highest level of antioxidants and carrots and chia seeds the lowest. Among the leafy greens tested, Figure 6, kale had the highest levels of antioxidants and spinach the least.

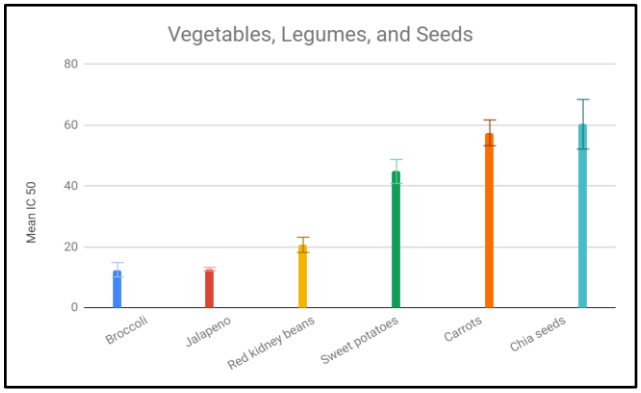

Figure 5. Shows antioxidant capacity of vegetables, legumes, and seeds

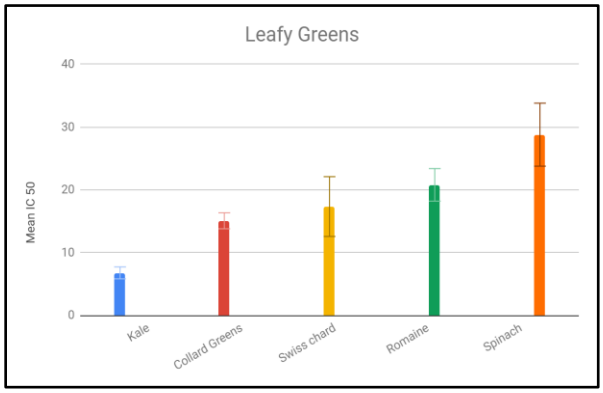

Figure 6. Shows antioxidant capacity of leafy greens

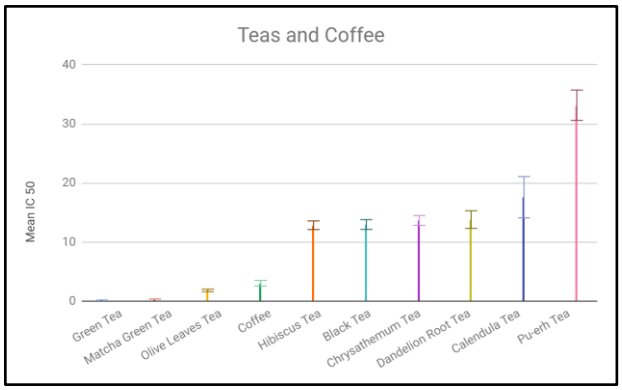

Figure 7. Shows antioxidant capacity of teas and coffee

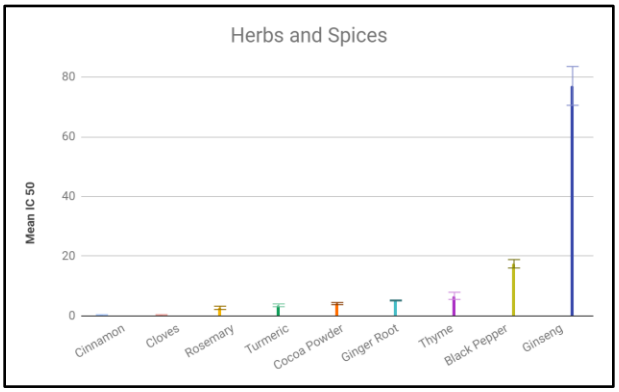

Figure 8. Shows antioxidant activity of herbs and spices

Among teas and coffee tested, Figure 7, green tea and matcha tea, a green tea powder, were the most reactive compounds tested. Olive leaves tea and coffee were also rich in antioxidants. Although black tea and green tea are derived from the same plant, black tea undergoes a fermentation process that causes a drop in its antioxidant potency. Pu-erh Tea, a fermented tea from China, had the lowest level of antioxidants. In the herbs and spices category, Figure 8, cinnamon and cloves outcompeted the rest of the spices tested. Black pepper and ginseng turned out to have the least antioxidant capacity of all the spices.

4. Conclusion

There are different avenues that can be utilized to adequately measure the scavenging ability of antioxidants. In addition to the DPPH assay that was used for the current study, assays of ABTS, FRAP, ORAC, among others, may also be used. Similar to the DPPH assay, the ABTS free radical (2,2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid) has a comparative mode of action. ABTS is a stable free radical that displays a blue color and absorbs at 734 nm in water.[4] In the presence of an antioxidant, the ABTS free radical will show a change in color which may be used to quantify the percent inhibition at different concentrations. An advantage of the ABTS assay has to do with its ability to take into account the pH of the antioxidant extract.[8] The FRAP assay focuses in on the ferric ion reducing antioxidant power. To quantify the antioxidant capacity, the FRAP assay uses a low pH buffer to measure the reduction of the ferric tripyridyltriazine complex (Fe3+) to a ferrous (Fe2+) form. The ferrous (Fe2+) form will display a color development compared to the Fe3+ complex, indicating the presence of antioxidants.[9] The FRAP assay concentrates on an antioxidant capacity to undergo a one-electron redox reaction. That said, the FRAP assay will exclude any other antioxidants contained in the extract that may scavenge free radicals via a different mode of action.[7] Previous studies have used the ORAC assay (oxygen radical antioxidant capacity) when working with fruit extracts.[10] The ORAC assay utilizes a fluoroscein probe capable of measuring the fluorescent signal an antioxidant can produce in the presence of a free radical. Advantages of this method include the ability of measuring both hydrophilic and hydrophobic characters of tested compounds as well as their full antioxidant capacity.[11]

In the current study, DPPH assay was deemed an appropriate measurement tool for the antioxidant activity of a variety of vegetables, herbs, spices, and natural beverages. The DPPH protocol takes into account the concentration of the antioxidant extract, the reaction time, and the temperature at which the reaction is being completed (room temperature ~ 23°C). The conditions listed can be controlled before-hand, which makes the DPPH assay reliable and the results reproducible.[12] Disadvantages of the DPPH assay relate to the natural pigmentation of certain tested compounds that may interfere with the absorption. Known compounds that could interfere, such as berries and tomatoes were not investigated in this study.

This investigation used the calculation of IC_{50} - the concentration at which 50% inhibition of the DPPH radical absorbance is observed. The more reactive a compound, the smaller its corresponding IC_{50} value. According to the findings above, the most reactive compounds belong to the natural beverage and spice categories. Green tea, cinnamon, and cloves are among the most reactive compounds tested with the smallest IC_{50} values. On the other end of the spectrum, the least reactive compounds include carrots, chia seeds, and ginseng. These compounds registered the largest IC_{50} values. Several foods tested are known for containing fat-soluble vitamins, such as vitamins A, D, E, and K which are known to have antioxidant behavior. Carrots and sweet potatoes owe their orange pigment to the presence of beta-carotene, an antioxidant that serves as the precursor to vitamin A in the human body. Although not orange in color, spinach has been known to contain beta-carotene as well.[13] The solubility of these hydrophobic compounds and the hydrophilic 80% ethanol solvent used may explain the high IC_{50} found in carrots, sweet potatoes, and spinach despite containing the antioxidant beta-carotene.

The findings of this study demonstrate the vast presence of antioxidants in commonly consumed foods and beverages. Future research in this area will focus on how serving sizes, various cooking methods and solvent used will impact the antioxidant potency of each studied food.

5. Acknowledgements

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6. References

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