

# Measurement of Antioxidant Activity in Selected Food Products and Nutraceuticals

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## Abstract

Measurement of antioxidant activity using *in vitro* assays is paramount in the evaluation of various food products and nutraceuticals for determining antioxidant benefits. This relates to the fact that increased antioxidant levels can protect the human body from free radical damage. The objective of this study was to quantify the antioxidant activity of selected food products and nutraceuticals using a spectrophotometric assay. Using Efferdent tablets and isotonic saline diluent, antioxidant levels could be determined by the amount of FD & C Blue No. 2 remaining after incubation at 37°C for 25 minutes. The antioxidant activity of a 500 mg quantity of vitamin C (ascorbic acid) was used as standard. The results obtained indicated that agave nectar syrup and xylitol sweetener (in descending order) exhibited higher antioxidant properties than all the other commercial sweeteners tested. Grapefruit was found to have the highest antioxidant activity of those common citrus fruits tested. Popular contemporary antioxidant beverages, such as strawberry-kiwi blend tend to have very high antioxidant activity since they contain d-alpha tocopheryl acetate (a form of vitamin E). Among the herbal nutraceuticals tested, spirulina and red yeast rice exhibited abundant antioxidant activities, whereas graviola was found to have absolutely no antioxidant properties. Thieves' oil was observed to have high antioxidant properties due to the synergism of the five oils in the preparation. Test results indicated a variable degree of antioxidant activity in foods and nutraceuticals compared to a standard antioxidant of 500 mg of vitamin C (ascorbic acid). Citrus fruits occupy the highest level of antioxidant activity overall, followed by antioxidant containing beverages and selected herbal products.

**Keywords:** Antioxidant; Sweeteners; Citrus fruits; Beverages; Nutraceuticals; Vitamin-C

## Introduction

Free radicals are atomic or molecular species containing one or more unpaired electrons that can exist freely in solution [1]. Although free radicals play a significant role in biological systems, they may cause extreme damage to biomolecules when present in excessive amounts [2,3]. Antioxidant activity can be defined as the protection against and repair of oxidative damage [4]. Antioxidants can reduce the risk for chronic diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, and immune dysfunction [5]. Components of the enzymatic antioxidant system include: superoxide dismutase; catalase; and glutathione peroxidase (GPx). The role of these enzymes is to convert free radicals into non-reactive species [6]. Nutraceutical foods provide medical or health benefits, including the prevention and treatment of diseases [7]. Nutraceutical is a "portmanteau" word, a combination from the two words "nutrition" and "pharmaceutical" and refers to the study of foods that have beneficial effects on human health. These compounds have great potential in the emerging nutritional industry, because they are often considered as food and medicines as well, therefore they may be used in the prevention and curative treatments. Epidemiological studies have shown that the consumption of a diet rich in fruit and vegetables is associated with decreased risk of developing certain chronic diseases, including heart disease, muscular degeneration and cancer [8,9]. Vitamin C can reduce the risk for cardiovascular disease by protecting LDL-cholesterol from oxidation [10]. Additionally, vitamin C exhibits significant antioxidant function in the lungs, protecting the rest of the body from exogenous sources of free radicals. Furthermore, when vitamin E is oxidized, vitamin C regenerates vitamin E back to its active form.

The richest source of vitamin C is citrus fruit, such as lemons, limes, oranges and grapefruit. One study conducted on a small population of smokers suggested that vitamin E may help in lowering the incidence of cardiovascular deaths in these individuals [11]. Beta-carotene is a

fat-soluble antioxidant, and a provitamin (precursor of vitamin A). Dark-green leafy vegetables are a rich source of carotenes [12]. Over 600 phytochemicals, including flavonoids and phenols, identified to date, are unique to plants [13]. Sources of these phytochemicals are: tea, garlic, oils, fruits, and vegetables. In general, the antioxidant activity of phytochemicals is 10-fold greater than the antioxidant activity of vitamins found in animal diets [14]. The antioxidant activity of various foods and dietary supplements is an important subject for investigation. Detailed information about the health promoting components of nutraceuticals could lead to a better understanding of the beneficial effects and an increased consumption of these fruits, including their utilization in functional foods and as ingredients in medicine and pharmaceuticals [15]. The potential of antioxidants to lower the risk of chronic diseases by scavenging harmful free radicals *in vivo*, forces us to evaluate the antioxidant activity of foods and nutraceuticals by using simple assays adaptable to the nutrition laboratory which are convenient, accurate, and rapid to perform. The objective of this study was to quantify the antioxidant activity of selected food products and nutraceuticals using a spectrophotometric assay.

## Materials and Methods

An interesting method for the qualitative evaluation of antioxidant activity is presented in the Certified Herbal Counselor Course

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Instruction Manual, JLS Educational Services [16]. The instructions require that 3 ounces of saline be placed into glass beakers. A vitamin C tablet (500 mg) is added to one of the container. Then an Efferdent tablet, containing a blue dye, is added to each container. The reaction is observed for 25 minutes and the change in blue color intensity is noted after 25 minutes. The glass beaker containing no antioxidant activity (no vitamin C) should lose most of its blue color. The darker the remaining blue color of the solution tested, the more antioxidant activity is associated with the sample.

The Efferdent tablet when dissolved in saline causes the sodium perborate monohydrate to form  $H_2O_2$ . The  $H_2O_2$  reacts with a catalyst (contained in the Efferdent tablet) to form  $O_2$  and  $H_2O$ .  $BNaO_3 \cdot H_2O$ . The chemicals in Efferdent act as bleaching agents ( $H_2O_2$ ,  $OH^-$ , and  $O^-$ ) to remove the discoloration of stains on dentures. Potassium peroxymonosulfate ( $KHSO_5$ ) also bleaches stains and disinfects. FD & C Blue No. 2 colors (also known as indigotine) the freshly dissolved Efferdent/saline solution dark blue. If left undisturbed for 25 to 30 minutes, the dark blue solution will lose its blue color.

If antioxidants, in the form of antioxidant containing food products, are added to a beaker containing 80 ml of a dissolved Efferdent tablet in saline, any free antioxidants in the food product will neutralize free radicals formed by the dissolution of the Efferdent tablet, with the result that a proportional amount of FD & C Blue No. 2 will not be oxidized to a colorless form. If, after 25 minutes, the resultant blue coloration of the Efferdent solution is measured spectrophotometrically at the optimum absorbance wavelength for FD & C Blue No. 2 (~ 610 nm), this original qualitative assay can be converted to a quantitative estimate of antioxidant activity. Relating the absorbance of the amount of blue color remaining in the Efferdent/saline solution after 25 minutes to a similarly treated Efferdent/saline solution containing 500 mg of pure ascorbic acid (Vitamin C) will allow for a standardized unit of antioxidant activity (herein termed the "Mesfer" unit) to be established for each antioxidant-containing food product assayed. This standardized unit will easily allow for comparison studies that can gauge the relative antioxidant activities of selected food products standardized to that of a 500 mg quantity of vitamin C.

For the preparation of the test samples, 80 ml of isotonic saline solution was added to the recommended serving size of each sample in separate beakers. Each test sample was gently mixed using an applicator stick until dissolved. All the glass beakers were maintained at 37°C (body temperature) on a hot plate. An Efferdent tablet was added to each glass beaker, and then gently mixed with an applicator stick until it dissolved. All the glass beakers were incubated for 25 minutes.

The primary aim of this study is to quantify the antioxidant activity of selected food products and nutraceuticals (including phytochemicals) by using a spectrophotometric assay. While a diverse array of antioxidants have been found in natural substances, their activities have been evaluated primarily by qualitative assays. Among the widely used assays measuring antioxidant activity, a spectrophotometric assay was selected as the most appropriate to provide quantitative data by a simple procedure using readily available supplies and instrumentation at the Rochester Institute of Technology, NY, USA.

The foods and nutraceuticals that were selected in this present study were chosen for their potential antioxidant properties. These contained vitamin A, C, and E, beta-carotene, and polyphenolic compounds. Moreover, they are commonly consumed in the American diet.

The sweeteners that were selected in this study were investigated for any potential antioxidant activity. Xylitol, for example, was chosen because of its association with significant improvement in dental health as well as blood sugar control. Citrus fruits and antioxidant beverages were also selected because of their historic antioxidant properties. A few popular herbal products were also selected for this study since they are believed to be rich sources of phytochemical antioxidants. Policosanol is a mixture of phytonutrients that are extracted from the outside of stalk sugar cane (*Saccharum officinarum*). This active substance is believed to lower cholesterol levels by inhibiting the formation of cholesterol in the liver. Another selected substance was red yeast rice, which is commonly used in Chinese traditional medicine. It is a substance derived from rice that is fermented with a type of yeast known as *Monascus purpureus*. It is believed to lower blood cholesterol levels by acting like lovastatin\*. Green tea is a herbal product, which is marketed on the basis of high antioxidant levels and because it has large amounts of (-) Epigallocatechin-3-gallate (EGCG). Spirulina is a blue-green algae. It is a plant-like organism that is commonly found in salt water and in some large fresh water lakes. This product is believed to boost the immune system. Curcuminoid-95+ is extracted from turmeric (*Curcuma longa*). It is thought to be rich in antioxidant, anti-inflammatory, and antimicrobial properties. Graviola (*Annona muricata*) is extracted from the graviola leaf in a base of super-oxygenated water and organic grain alcohol. It is traditionally used to treat inflammatory conditions. Furthermore, a study performed at National Cancer Institute found graviola extract to be an effective anti-cancer agent [17].

Lastly, Thieves' oil was selected for testing its antioxidant activity. Thieves' oil was invented by four thieves in France during medieval times. They combined oils of clove, rosemary, cinnamon, lemon, and eucalyptus to protect themselves from the plague while robbing dead victims. Each of the Thieves' oil constituents may have antioxidant properties. Clove oil is an abundant source of eugenol, which has high antioxidant content. Oil that is a powerful antioxidant is cinnamon oil. It also plays a significant role in supporting the digestive system and in controlling blood sugar levels. Rosemary oil is also present in Thieves' oil and is believed to help restore mental alertness once fatigue occurs. With 68% d-limonene, lemon oil has effective antioxidant properties. It also works as a solvent and cleanser. Eucalyptus oil has many health functions. It contains eucalyptol, which is reported to efficiently fight bacteria [18]. Synergetic effect of Thieves' oil was investigated by combining all five oils. The individual oils were also studied by using the spectrophotometric assay.

## Results

The absorption spectrum of dissolved Efferdent reveals the maximum absorbance to be at 610 nm (Figure 1). The antioxidant activity of all the substances tested is presented in table 1.

### Antioxidant activity of sweetener compared to 500 mg of vitamin C

The result of the study on the antioxidant activity of sweetener compared to 500 mg of vitamin C are represented in figure 2. The mean antioxidant activity compared to 500 mg vitamin C (taken as 1.000 Mesfer unit-MU) for aspartame, sucralose, saccharin, xylitol, stevia, erythritol, raw sugar, pure cane sugar, agave nectar syrup and pure maple syrup were 0.284, 0.164, 0.118, 0.300, 0.013, 0.170, 0.0000, 0.000, 0.900 and 0.067 respectively. It is worth mentioning that agave nectar

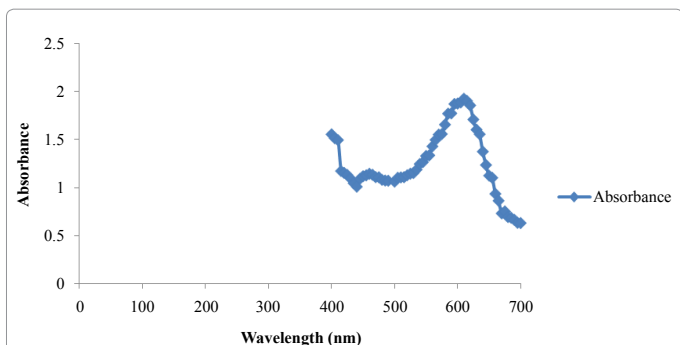


Figure 1: Showing the maximum absorbance of Efferdent tablet at 610 nm.

| Antioxidant                  | Absorbance | Antioxidant activity (Mesfer Unit) |
|------------------------------|------------|------------------------------------|
| <b>Ascorbic Acid</b>         | 1.700      | 1.000                              |
| <b>SWEETENERS</b>            |            |                                    |
| Aspartame                    | 0.483      | 0.284                              |
| Sucralose                    | 0.279      | 0.164                              |
| Saccharin                    | 0.202      | 0.118                              |
| Xylitol                      | 0.510      | 0.300                              |
| Stevia                       | 0.023      | 0.013                              |
| Erythritol                   | 0.289      | 0.170                              |
| Raw sugar                    | 0.000      | 0.000                              |
| Pure cane sugar              | 0.000      | 0.000                              |
| Agave nectar syrup           | 1.531      | 0.900                              |
| Pure maple syrup             | 0.115      | 0.067                              |
| <b>CITRUS FRUITS</b>         |            |                                    |
| Orange                       | 2.830      | 1.664                              |
| Lime                         | 2.080      | 1.223                              |
| Lemon                        | 2.395      | 1.408                              |
| Grapefruit                   | 2.853      | 1.678                              |
| Ugli fruit                   | 2.091      | 1.230                              |
| Goji berry                   | 1.435      | 0.844                              |
| <b>BEVERAGES</b>             |            |                                    |
| 7UP Mixed Berry Antioxidant  | 1.619      | 0.952                              |
| Cranberry Energy Juice Drink | 1.783      | 1.048                              |
| Blueberry Green Tea          | 1.813      | 1.066                              |
| Coconut Water, 100% Pure     | 1.002      | 0.589                              |
| Energy Drink                 | 1.667      | 0.980                              |
| Strawberry Kiwi              | 2.921      | 1.718                              |
| Cherry 7Up Antioxidant       | 2.077      | 1.221                              |
| <b>NUTRACEUTICALS</b>        |            |                                    |
| Policosanol                  | 0.945      | 0.555                              |
| Red Yeast Rice               | 1.814      | 1.067                              |
| Green Tea Concentrate        | 0.725      | 0.426                              |
| Spirulina                    | 5.050      | 2.970                              |
| Graviola                     | 0.000      | 0.000                              |
| Curcuminoid-95+              | 0.634      | 0.372                              |
| <b>THIEVES' OIL</b>          |            |                                    |
| Rosemary                     | 0.000      | 0.000                              |
| Eucalyptus                   | 0.000      | 0.000                              |
| Quantum Limonene Complex     | 1.114      | 0.655                              |
| Cinnamon Oil                 | 0.553      | 0.325                              |
| Clove bud                    | 0.325      | 0.191                              |

Table 1: Absorbance and activity of various antioxidants as compared to vitamin C (ascorbic acid).

syrup can be compared with vitamin C because of its high antioxidant capacity.

### Antioxidant activity of selected citrus fruits compared to 500 mg of vitamin C

The result of the study on the antioxidant activity of selected citrus fruits compared to 500 mg of vitamin C are represented in figure 3. The mean antioxidant activity compared to 500 mg vitamin C (taken as 1.000 Mesfer unit-MU) for orange, lime, lemon, grapefruit, ugli fruit and goji berry were 1.664, 1.223, 1.408, 1.678, 1.230 and 0.844 respectively. It is worth mentioning that all the selected citrus fruits possessed high antioxidant capacity.

### Antioxidant activity of selected beverages compared to 500 mg of vitamin C

The result of the study on the antioxidant activity of selected beverages compared to 500 mg of vitamin C are represented in figure 4. The mean antioxidant activity compared to 500 mg vitamin C (taken as 1.000 Mesfer unit-MU) for 7 up mixed berry, cranberry energy juice, blueberry green tea, coconut water (100% pure), energy drink,

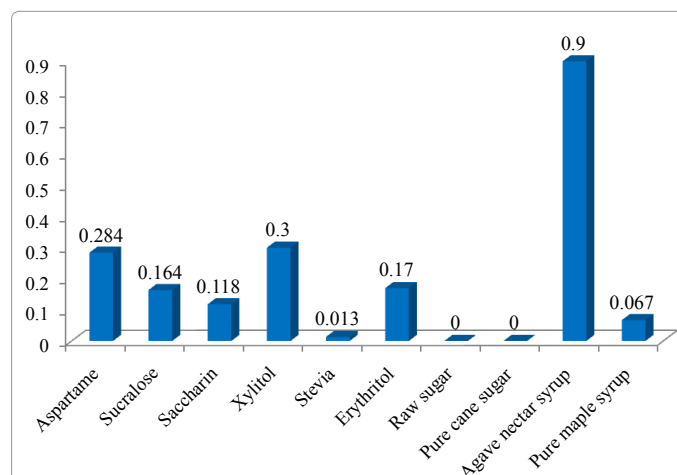


Figure 2: Antioxidant absorbance and activity values for selected sweeteners as compared to ascorbic acid (=1.000 MU).

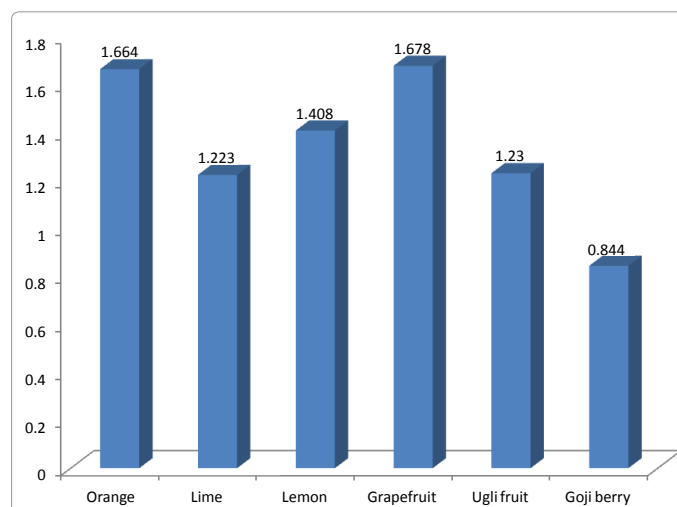


Figure 3: Antioxidant absorbance and activity values for selected citrus fruits as compared to ascorbic acid (=1.000 MU).

strawberry kiwi and cherry 7 up antioxidant were 0.952, 1.048, 1.066, 0.589, 0.980, 1.718 and 1.221 respectively.

### Antioxidant activity of selected nutraceutical products compared to 500 mg of vitamin C

The result of the study on the antioxidant activity of selected nutraceutical products compared to 500 mg of vitamin C are represented in figure 5. The mean antioxidant activity compared to 500 mg vitamin C (taken as 1.000 Mesfer unit-MU) for policosanol, red yeast rice, green tea concentrate, spirulina, graviola and curcuminoid-95+ were 1.032, 0.802, 1.290 and 0.787 respectively.

### Antioxidant activity of the constituents of thieves' essential oil compared to 500 mg of vitamin C

The result of the study on the antioxidant activity of thieves' essential oil compared to 500 mg of vitamin C are represented in figure 6. The mean antioxidant activity compared to 500 mg vitamin C (taken as 1.000 Mesfer unit-MU) for rosemary, eucalyptus, qanatum+limonene complex, cinnamon oil, clove bud and rose mary+eucalyptus+limonene+cinnamon+clove were 0.000, 0.000, 0.655, 0.325, 0.191 and 0.817 respectively.

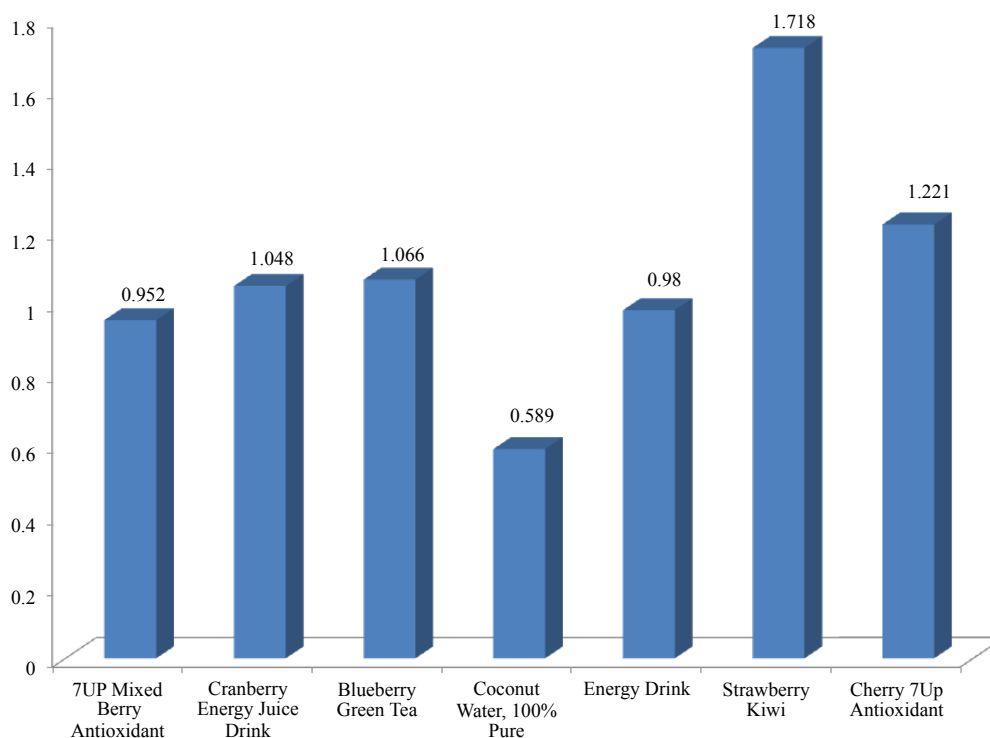


Figure 4: Antioxidant absorbance and activity values for selected antioxidant beverages as compared to ascorbic acid (=1.000 MU).

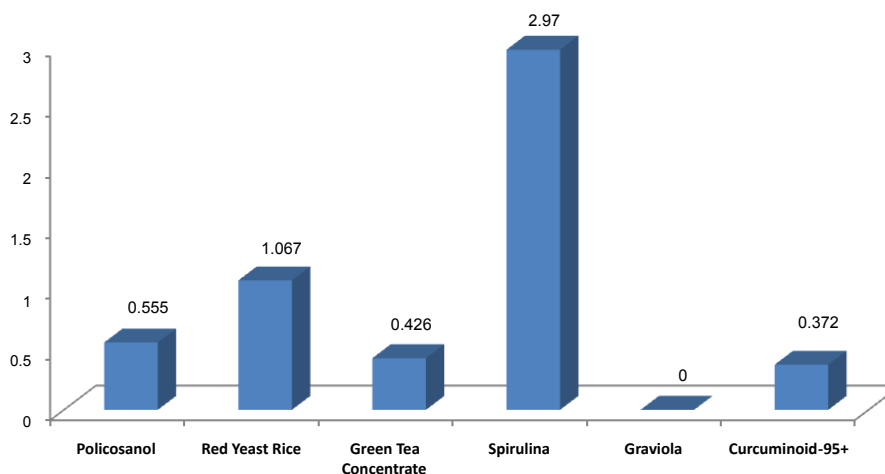
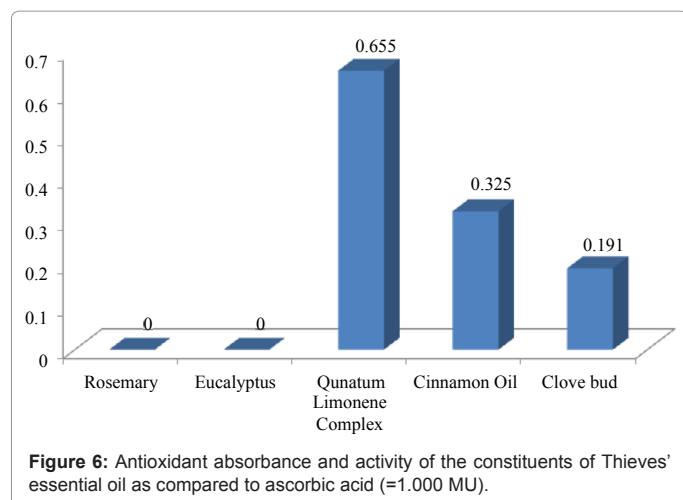


Figure 5: Antioxidant absorbance and activity values for selected nutraceutical products as compared to ascorbic acid (=1.000 MU).





## Discussion

The antioxidant activity of 500 mg of vitamin C (ascorbic acid) was used as the standard for comparative purposes, as presented in table 1. Recent nutritional research has focused on the antioxidant potential of foods, while current dietary recommendations are to increase the intake of antioxidant-rich foods rather than supplement specific nutrients. Many alternatives to refined sugar are available, including raw cane sugar, plant saps/syrups (e.g. maple syrup, agave nectar), molasses, honey, and fruit sugars (e.g. date sugar). Unrefined sweeteners were hypothesized to contain higher levels of antioxidants, similar to the contrast between whole and refined grain products [19]. It was observed that agave nectar syrup and xylitol sweetener (in descending order) had higher antioxidant properties than all the other common sweeteners tested. Among all the citrus fruits tested, grape fruit demonstrated the highest antioxidant activity per standard serving size; this may be due to the presence of high amounts of vitamin C, lycopene, and the soluble pectin in it [20]. Antioxidant activities of selected antioxidant beverages indicated that strawberry-kiwi blend demonstrates the highest antioxidant levels among the selected antioxidant drinks. This high antioxidant activity is probably attributable to the high content of vitamin C, carotenoids and d-alpha tocopheryl acetate (a form of vitamin E) in strawberry and in kiwi. Because of the fairly high content of antioxidants and the frequent use, coffee and tea are important antioxidant sources in many diets. Several different compounds contribute to coffee's antioxidant content, e.g., caffeine, volatile aroma compounds and heterocyclic compounds [21-24]. Many of these are efficiently absorbed, and plasma antioxidants increase after coffee intake [25]. In green tea, the major flavonoids present are the monomer catechins, epigallocatechin gallate, epigallocatechin, epicatechin gallate and epicatechin. In black tea the polymerized catechins, the aflavin and the arubigen predominate in addition to quercetin and flavonols [26,27]. Many herbs and spices are the subject of ongoing scientific investigations related to antioxidant properties and health. Epidemiological evidence exists indicating that there is a correlation between increased dietary intake of antioxidants and a lower incidence of morbidity and mortality [28]. For instance, a population-based case-control study in approximately 500 newly diagnosed gastric adenocarcinoma patients and approximately 1100 control subjects in Sweden found that the total antioxidant potential of several plant-based dietary components was inversely associated with gastric cancer risk [29]. The largest published study to date which tested the antioxidant activity of foods from a nationally representative food

evaluated the antioxidant activity in both water-soluble and fat soluble fractions of 1,113 food samples from the USDA's National Food and Nutrient Analysis Program (NFNAP) using the FRAP method [30]. This study found that of the top 50 foods with antioxidants, the top five antioxidants were dried spices (ground cloves, dried oregano, ground ginger, ground cinnamon, turmeric powder); however, compared to other categories of food products within this study, herbs and spices displayed the largest range in antioxidant capacity, 0.803-125.549 mmol/100 g [31]. This study also evaluated the antioxidant activities of selected natural herbal products. Among the selected herbal products tested, spirulina demonstrated the highest level of antioxidant activity (2.970), followed by red yeast rice (1.067). Graviola (an herbal anti-cancer extract) was found to have no antioxidant activity. The constituents of 'Thieves' oil blend, a combination of five essential oils that enhance the immune system, were also examined. Limonene, cinnamon, and clove oils were shown to possess high antioxidant activities, whereas rosemary and eucalyptus oil did not demonstrate any antioxidant activities by themselves. A prior study showed that limonene, cinnamon, and clove oils had all demonstrated high antioxidant effects on the oxygen radical absorbance capacity (ORAC) scale [1]. Therefore, spectrophotometric assay-based examination of the antioxidant activities of these oils provided further evidence that these oil blends have high antioxidant properties. Furthermore, a highly synergistic effect exists when all five essential oils are blended together into "Thieves" oil. Much remains to be done to understand the mechanisms of action for antioxidants, their impact on various types of tumors, the degree to which antioxidants are absorbed from foods, and what effective concentrations are needed in humans to reduce oxidative stress at the tissue or cellular level and how the antioxidant capacity of foods and food components relates to physiologic events in humans. To facilitate research in this area, there remains a need to collect additional data on the antioxidant capacity of herbs, spices, and their bioactive components from *in vivo*, *in vitro*, and clinical studies and establish more detailed research databases to serve as a repository for this data from a variety of disciplines to promote trans-disciplinary research and ultimately aid in fostering new discoveries.

## Conclusion

As a result of the potential of antioxidants to lower the risk of chronic diseases by scavenging harmful free radicals *in vivo*, it is important to be able to evaluate antioxidant activity of foods and nutraceuticals by using simple assays adaptable to the nutrition laboratory. In this study, it was shown that the antioxidant activities of selected foods and nutraceuticals could be measured conveniently, accurately, and rapidly using this spectrophotometric assay. Test results indicated a variable degree of antioxidant activity in foods and nutraceuticals compared to a standard antioxidant of 500 mg of vitamin C (ascorbic acid). Citrus fruits occupy the highest level of antioxidant activity overall, followed by antioxidant containing beverages and selected herbal products. These results suggest that the antioxidant activity of these foods contribute to a reduction in the risk of free radical generation and subsequent damage to human cells and tissues.

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