SELECTED FOOD SUPPLEMENTS AND THEIR TOTAL FLAVONOIDS, TOTAL PHENOLICS AND VITAMIN C AS MEASURES OF EFFECTIVENESS AGAINST OXIDATIVE STRESS

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Abstract— Food supplements have become a part of consumer market preferences over the past years mainly as alternative medicine and as source of nutrients that contributes to overall body health and vigor. The present investigation has been carried out to evaluate and compare the antioxidant activities based on the levels of the total flavonoids, total phenolics, and vitamin C of ten selected food supplements. The food supplements included in this study are labeled as ACN, BME, CSH, DTH, EAP, FNC, GYK, HAV, IPL, and JGP. Supplement ACN is derived from a wide variety of fruits, vegetables, and herbs; Supplement BME is from mangosteen (*Garcinia mangostana)*; Supplement CSH is made from herbal plants and ginseng roots; Supplement DTH obtained solely from the inner bark of the Red Lapacho tree (*Tabebuia heptophylla)*; Supplement EAP is a blend of ampalaya (*Momordica charantia*), banaba *(Lagerstroemia speciosa)* and luyang dilaw (*Curcuma longa)*; Supplement FNC is prepared from malunggay (*Moringa oleifera)*; Supplement GYK is derived from blue green algae, ampalaya (*Momordica charantia);* malunggay (*Moringa oleifera);* sambong *(Lumae balsamifera)*; garlic (*Allium sativum*); Supplement HAV is a combination of variety of fruits and vegetables; Supplement IPL is derived solely from yellow trumpet tree (*Tabebuias tree*); and supplement JGP is made from fennel *(Foeniculum vulgare)*; ginger *(Zingiber officinale-rhizome)*; mustard seed *(Brassica alba)* and garlic *(Allium sativum).*
The results for total flavonoids ranged from 10.05 to 96.75 milligrams quercetin per gram of dried material with the highest value for Supplement BME and lowest for Supplement ACN. The results for total phenolics varied from 2.84 to 37.71 mg GAE per g dried material with the highest value for Supplement BME and the lowest for Supplement CSH. The vitamin C contents varied from 3.52 to 47.86 mg ascorbic acid per g dried material with the highest value for Supplement HAV and the lowest for Supplement IPL. The overall results demonstrated that these selected food supplements are rich sources of natural antioxidants at varying degrees and may be promising against various types of chronic and degenerative diseases caused by oxidative stress.

Keywords— Food Supplement, Antioxidant, Free Radicals, Phytochemicals, Total Flavonoids, Total Phenols.

I. INTRODUCTION

Oxygen is an essential element for life. Living systems have evolved to survive in the presence of molecular oxygen. Oxidative properties of oxygen play a vital role in diverse biological phenomena. However, though oxygen may be obligatory for life but it can also aggravate the damage within the cell by oxidative events (Mahantesh et al, 2012).

Reactive oxygen species (ROS) is a term which encompasses all highly reactive, oxygen-containing molecules, including free radicals. It includes the hydroxyl radical, the superoxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various lipid peroxides. All are capable of reacting with membrane lipids, nucleic acids, proteins and enzymes, and other small molecules, resulting in cellular damage (Percival,1998).

Free radicals are continuously forming in cell and environment through UV radiations, X-rays, gamma rays and microwave radiation, metal-catalyzed reactions, oxygen free radicals in the atmosphere considered as pollutants, interaction with chemicals, automobile exhausts fumes, smoking of cigarettes burning of organic matter during cooking, forest fires, volcanic activities and industrial effluents, excess chemicals, alcoholic intake, certain drugs, asbestos, certain pesticides and herbicides, some metal ions, fungal toxins and xenobiotics (Dirks & Faiman,1982).

Several human diseases have been suspected to be caused by free radicals (Halliwell & Gutteridge, 1997) and according to Seis,1992 it appears to be a major contributor to aging and to degenerative disease of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. They are also capable of attacking the healthy cells of the body, causing them to lose their structure and function (Percival,1998).

To protect the cells and organ systems of the body against reactive oxygen species, and to prevent cells and organ systems for further damage, human has evolved a highly sophisticated and highly advance antioxidant defense system. Antioxidant system involves a variety of components, both endogenous and exogenous in origin, that function interactively and synergistically to neutralize free radicals (Jacob,1995).

Antioxidants are highly capable agents to fight against free radicals. They promote growth of healthy cells, protect cells against diseases and most importantly, they provide excellent support for the body's immune system (Chakraborty, Kumar, Dutta

& Gupta, 2009). A large body of literature has documented the beneficial effects of antioxidants in the human body, its role on scavenging free radicals, in the prevention and therapy and treating serious life threatening diseases (Barrett, 2003).

Antioxidant formulations are derived from variety of ingredients, including vitamins (tocopherols, ascorbic acid), bioactive compounds of plant origin (polyphenols and carotenoids, flavonoids) plant and algae extracts, fruits and vegetables concentrates, enzymes, minerals (selenium, zinc, manganese), polysaccharides, organosulfur compounds(Almeida et al, 2011).

Recently, plant-derived substances have become everyone's great passion due to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs and food supplements (Ncube, Afolayan & Okoh, 2008), which contain complex mixture of many medicinal plant metabolites, such as alkaloids, glycosides, terpenoids, flavonoids and lignans which are known as phytochemicals.

Phytochemicals, which are sometimes referred to as phytonutrients exhibit diversified physiologic and pharmacologic effects. They have been found to inactivate cancer-causing substances, stimulate the immune system, protect the heart from disease, and help prevent cataracts. They promote impact on health as antioxidants, blood pressure or blood sugar influencing substances, antibacterial, antifungal, antiviral, cholesterol-lowering, antithrombotic or anti-inflammatory effects (Watzl & Leitzmann, 1999). Furthermore, it can promote health by strengthening the blood vessels, fighting tumors, and through other activities. Many of these non-nutritive substances have potent biological activity and may help to lower risk for many chronic diseases. Groups of these functional bioactive compounds consists a wide range of substances. They are classified according to their physiological and pharmaceutical effects.

Flavonoids are water soluble polyphenolic molecules containing 15 carbon atoms. They belong to the polyphenol family. flavonoids consist of 6 major subgroups: chalcone, flavone, flavonol, flavanone, anthocyanins and isoflavonoids. Together with carotenes, flavanoids are also responsible for the coloring of fruits, vegetables and herbs. These are the most diverse group of phytochemicals of naturally occurring benzo-g-pyrone derivatives, possess several biological properties (including hepatoprotective, anti-thrambotic, antiinflammatory, and antiviral activities), many of which may be related, partially at least, to their antioxidant and free-radical-scavenging ability. (Saija, Scalese, Lanza, Marzullo, Bonina, & Castelli, 1995).

They are known to have antiradical property which is directed mostly toward HO; and 0_2 - as well as peroxyl and alkoxyl radicals. These compounds present a strong affinity for iron ions which are known to catalyze many processes leading to the appearance of free radicals. They are effective on scavenging oxygen-derived free radicals. Possess antiinflammatory, antiallergic, antiviral, and anticarcinogenic properties. They have highly significant antioxidant activity and are known to have health promoting effects like anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral. The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma. (Saija et al, 1995).

Flavonoids, or bioflavonoids, are universal group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory actions. They have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals. (Miller,1996).

Food supplements are a type of dietary supplement that contain herbs, either singly or in mixtures. They are taken by mouth that contains a dietary ingredient intended to supplement the diet like vitamins, minerals, herbs or other botanicals, amino acids, and substances such as enzymes, organ tissues, glandulars, and metabolites.

These supplements are distributed as isolated substances or as mixtures, from natural or synthetic origin, extracts or concentrates presented in a variety of forms including pills, powders, drinks, supplement bars, tablets, capsules, softgels, gelcaps and liquids (Kunwar & Priyadarsini,2011).

They are found at most grocery, convenience and wellness stores which provide benefits for people experiencing nutrient deficiencies, although others hold risks for serious side effects. It provides a convenient means of attaining vitamins, minerals and other nutrients normally derived from food. They may fill in the gaps when people have nutrient deficiencies due to medical conditions and support nutritional wellness in those unable to attain proper nutrients through food sources. People may seek these supplements for an array of reasons, such as to improve digestion, relaxation, energy or immune system function, or as a means of managing appetite or body weight. (McLaughlin, 2010).

Many studies have shown the presence of herbal compounds in food supplements which play powerful roles in preventing, relieving and treating serious life threatening diseases such as Acquired Immunodeficiency Syndrome (AIDS), as well as their utility in more practical applications such as immune-enhancement (Barrett, 2003).

In this research, the following supplements were studied. Their claims and components were included

as part of this present study. Its true identity were purposely withheld to protect the owner's rights.

- Supplement ACN is derived from a wide variety of fruits, vegetables, and herbs; helps fight against cancer and heart diseases, prevents blood clots and detoxify the liver and counter the negative effect of alcohol (such as liver damage). It have been found to contain carbohydrates, reducing sugars, saponins, flavonids and steroids.
- Supplement BME is from mangosteen (*Garcinia mangostana)*; help address health problems such as insomnia and asthma, improve the condition of patients suffering from cancer and tumor. It contains carbohydrates, reducing sugar, tannins, saponins, flavonoids and alkaloids.
- Supplement CSH is made from herbal plants and ginseng roots; help increase energy, improve digestion and promotes regular bowel movement. The main constituents of this supplement are carbohydrates, reducing sugar, saponins, flavonoids and alkaloids.
- Supplement DTH is obtained solely from the inner bark of the Red Lapacho tree (*Tabebuia heptophylla).* It is anti-microbial, anti-fungal and anti-bacterial. It contains active compounds like carbohydrates, reducing sugar, tannins, saponins, flavonoids and steroids
- Supplement EAP is a blend of ampalaya (*Momordica charantia*), banaba *(Lagerstroemia speciosa)* and luyang dilaw (*Curcuma longa).* It is used as a diuretic, known to lower blood sugar, helps in reducing weight has anti-inflammatory and anti-cancer properties, protect the liver and help maintain liver function. It is found to contain carbohydrates, reducing sugar, tannins, saponins, flavonoids, anthraquinones, steroids and alkaloids.
- Supplement FNC is prepared from malunggay (*Moringa oleifera).It* promotes milk secretion in mothers with inadequate lactation, and can cure inflammatory swelling. It contain carbohydrates, reducing sugar, saponins, flavonoids and steroids
- Supplement GYK is derived from blue green algae, ampalaya (*Momordica charantia);* malunggay (*Moringa oleifera);* sambong *(Lumae balsamifera)*; garlic (*Allium sativum*) for the treatment of mild and serious ailments. It contains carbohydrates, reducing sugar, tannins, saponins, and alkaloids.
- Supplement HAV is a combination of variety of fruits and vegetables. Its essential nutrients help support healthy bones, eyes, heart, colon, promotes daily energy and immune defense. It contains carbohydrates, saponins and alkaloids.
- Supplement IPL is derived solely from yellow trumpet tree (*Tabebuias tree*). It promotes and supports oxygenation and detoxification and degeneration of vital health to all parts of the body. It contain, reducing sugar, saponins, steroids and alkaloids.
- Supplement JGP is made from fennel *(Foeniculum vulgare)*; ginger *(Zingiber officinale-rhizome)*; mustard seed *(Brassica alba)* and garlic *(Allium sativum).*Helps prevents proliferation of cancer cells, increase energy and improve digestion. It contains carbohydrates, reducing sugar, tannins, saponins and alkaloids.

With an increasing number of food supplements in the market and with their therapeutic claims, it is best to confirm if such claims have empirical basis. In this present study, the antioxidant activity in terms of the total flavonoid content, total phenolic content and Vitamin C scavenging capacity of ten selected food supplements were evaluated.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

The researchers selected ten (10) supplements from different registered drugstores and pharmacy in Butuan City, Agusan del Norte, Philippines based on the consumers' preferences. The samples in capsule form were brought to Chemistry Laboratory of Mindanao University of Science and Technology, Cagayan de Oro City where the analysis was conducted. Each sample was removed from their respective capsules and were placed in air tight sealed containers ready for evaluation.

Preparation of Extracts. Corresponding amount of the samples were soaked with proportionate amount of methanol for 12 hours with occasional shaking. The mixtures were filtered separately and the filtrates were set aside. The residues were soaked with the same proportionate amount of methanol for another one hour, filtered and the filtrates were set aside. The residues were soaked further in an hour with the same amount of methanol, filtered and the filtrates were collected. The filtrates were combined and concentrated to about 100 mL using the rotary evaporator. The filtrate was then transferred in a volumetric flask, set aside for further analysis of solid content via oven method.

Determination of the concentration of the supplement extracts. Ten (10) ml of concentrated extract solution was placed into a pre-weigh aluminum dish. This was oven dried for 1 hour at a temperature three degrees (3^o) higher than the boiling point of methanol. The weight of the dish with the residue was measured. The same procedure was repeated until constant weight was obtained. From the resulting residue weight, the concentration was calculated. The concentration obtained was used to prepare the extract solution at a lower concentration of 1mg/mL for further testing protocols. The tests were subsequently done in 3 replicates.

UV-Vis Spectrophotometric Analysis for Total Flavonoid Content. The total flavonoid content of the

ten food supplement samples was determined with the aluminum chloride, $AlCl_3$ method using quercetin as the standard. The plant extract was pipetted out and added to 1.25 mL distilled water followed by 75µL of 5% $NaNO_2$. After five minutes at room temperature, 0.15mL of $AlCl_3$ solution was added. This was incubated for six minutes at room temperature to give time for the chemical reaction to take place. The reaction mixture was then treated with 0.5 mL of 1 mM NaOH. Then the reaction mixture was diluted with 275 µL of distilled water and incubated further for 20 minutes at room temperature. The absorbance was then read at 510 nm using UV-Vis spectrophotometer. The total flavonoid content was calculated from an absorbance-concentration calibration equation.

UV-Vis spectrophotometric analysis for total phenolic content. The determination of the total phenolic content was done using adopted procedure from Goyal, Middha, and Sen (2012) using the Folin-Ciocalteu (FC) reagent method with slight modification. Briefly, 2.0 mL of extract was mixed with 2.0 mL of FC reagent (previously diluted 1:1 with distilled water) and incubated for 5 minutes at room temperature. Then, 4 mL of 2% Na_2CO_3 solution was added. The absorbance was read at 730 nm after incubation for 10 min. Gallic acid monohydrate was used as the standard. The total phenolics was determined using an absorbance-concentration calibration equation and expressed as milligram of gallic acid equivalent (GAE) per gram dried material.

*Determination of vitamin C content***:** The vitamin C content of the ten samples were determined using the method reported by Benderitter et al. Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4.5H_2O$ in 100 mL of 5M H_2SO_4) was added to 500 µL reaction mixture (300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water). The reaction mixture was subsequently incubated for 3h at 37°C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was subsequently calculated from the calibration curve prepared with ascorbic acid standard.

III. RESULTS AND DISCUSSION

3.1. Total Flavonoid Content

Plants with flavonoids have been reported to have antioxidant activity and are known to have health promoting effect like anti-cancer, anti-microbial, anti-inflammation and anti-viral properties (Ghasemzadeh, Jaafar & Rahmat, 2010). The flavonoids quercetin is known for its ability to relieve hay fever, eczema, sinusitis and asthma (Saija, et al.,1995). Figure 1 shows the relative total flavonoid content of ten selected food supplements.

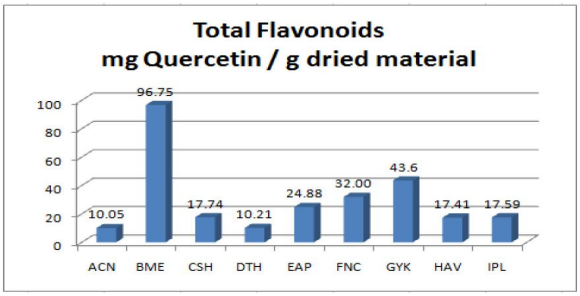


Figure 1: Total flavonoids of the ten selected food supplements.

Supplement BME which is derived solely from mangosteen confirmed to have the highest total flavonoid as shown in Figure 1, with an equivalent value of 96.75 mg quercetin per gram of dried material. Ranked second is *Supplement JGP*, derived from blend fennel, ginger, mustard seed & garlic with a concentration value of 48.06 mg quercetin per g dried material and *Supplement GYK*, from a mixture of blue green algae, ampalaya, malunggay, sambong & garlic ranked third with a concentration value of 43.60mg quercetin /g dried material.

An earlier study has shown that malunggay is rich in flavonoids (Ramamoorthy & Bono, 2011) and has been reported to be useful in treating diabetes, high blood pressure, cancer, eye problems and many other illnesses (Ying, West, Jensen, Nowicki, Chen, Palu, & Anderson, 2002).Plants with flavonoids, in general, have been reported to have a potent inhibitory effect on osteoclastogenesis and bone resorption. (Yamaquchi, Hamamoto, Uchiyama & Ishiyama, 2007). In addition, in recent studies, plants with flavonoids have been reported to have anti-cancer, anti-microbial and anti-inflammation properties (Ghasemzadeh, Jaafar & Rahmat,2010).

Total Phenolic Content.

Phenolic compounds have been reported to possess antioxidant activity which allows them to scavenge both active oxygen species and electrophiles (Rice-Evans, Miller, Bolwell, Bramley & Pridham, 1995). Its functions have been the subject of a great number of agricultural, biological, chemical and medical studies. The importance of antioxidant activities of phenolic compounds and their potential usage as a natural antioxidant compounds has reached a new level and that the biological actions of these compounds are related to their antioxidant activity (Gryglewski, Korbut & Robak,1987).

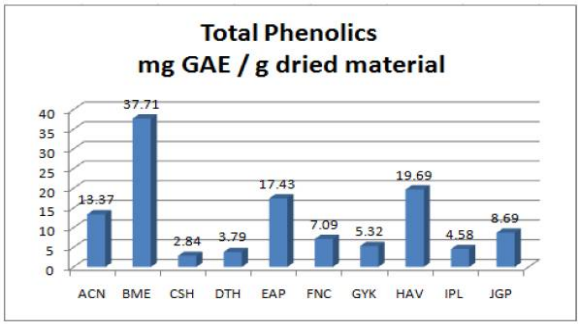


Figure 2: Total phenolics of the ten selected food supplements.

The average total Phenolic content of ten selected food supplements are presented in Figure 2. *Supplement BME* which is derived solely from mangosteen confirmed to have the highest total phenolic as shown in Figure 2, with an equivalent value of 37.71 mg GAE per gram of dried material. Ranked second is *Supplement HAV*, derived from variety of fruits & vegetables with a concentration value of 19.69. *Supplement EAP* which is made from a blend of ampalaya, banaba & luyang dilaw and *Supplement ACN* from wide variety of fruits , vegetables and herbs ranked third and fourth with equivalent values of 17.43 and 13.37 mg GAE/g dried material respectively. The rest of the supplements registered total phenolics of about 10 or lower.

Vitamin C

Vitamin C is an important antioxidant in human, capable of scavenging oxygen-derived free radicals (Afkhami-Ardekani,M & Shojaoddiny-Ardekani, A., 2007). Vitamin C is structurally similar to glucose and can replace it in many chemical reactions, and thus is effective in prevention of non-enzymatic glycosylation of proteins (Afkhami-Ardekani M, Vahidi AR, & Borjian,L., L.2003). In addition, vitamin C acts as a regulator of catabolism of cholesterol to bile acid in guinea pig and has been demonstrated to be an important factor in lipid regulation (Simom, JA.,(1992). Ness, et al showed beneficial effects of vitamin C on lipids in human. Figure 3 shows the relative Vitamin C content of ten selected food supplements.

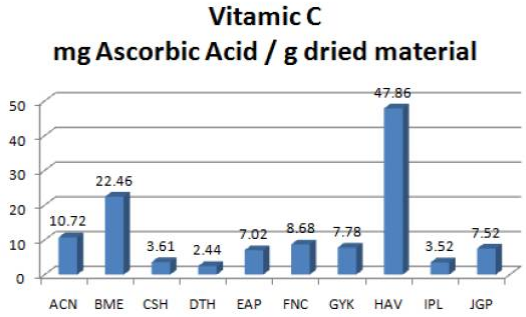


Figure 3: Vitamin C content of the ten selected food supplements.

The values as shown indicated that *Supplement HAV*, which is derived from variety of fruits & vegetables has the highest in vitamin C content with equivalent value of 47.86 mg Ascorbic Acid / g dried material followed by Supplement BME which contains mangosteen and Supplement ACN which is obtained from wide variety of fruits, vegetables and herbs with equivalent value 22.46 and 10.72 mg Ascorbic acid/g dried material as shown in figure 3 respectively. All other supplements were not so impressive in their vitamin C.

An earlier study has shown that Vitamin C, which is the most important vitamin for human nutrition that is supplied by fruits and vegetables, plays many crucial roles in growth and metabolism. (Hernández, Lobo, & González, 2000). As a potent antioxidant, Vitamin C has the capacity to eliminate several different reactive oxygen species, capable of maintaining the activity of a number of enzymes (by keeping metal ions in the reduced state), and has a role in stress resistance (Arrigoni & De Tullio, 2002; Davey et al., 2000; Klein & Kurilich, 2000).

CONCLUSIONS

In this study, BME registers the highest total flavonoids and total phenolics; it comes second in vitamin C content. On this basis, it may have superior antioxidant potential compared to the other food supplements. DTH appears to be a relatively poor performer in all 3 parameters measured. But it is clear that this and the other food supplements also have significant contents of the antioxidant substances studied, indicating that they are as well useful against oxidative stress and the resulting ailments at varying degrees. Antioxidant action is a complicated process and other tests can reveal more information that can help in coming up with a more complete picture.

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