Antioxidant Potential of Selected Indigenous Fruits Using *In Vitro* Lipid Peroxidation Assay

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This study was primarily concerned in determining the antioxidant potential of the edible parts of selected indigenous fruits grown in the Cordillera region namely ayosep (*Vaccinium angustifolium*), bitungol (*Flacourtia indica*), kalamondin (*Citrofortunella microcarpa*), marble tomato (*Lycopersicon pimpinellifolium*), native lacatan (*Musa paradisiaca*), native passion fruit (*Passiflora edulis*), pinit (*Rubus copelandii*), rattan (*Calamus mollis*), tamarillo (*Cyphomandra betacea*) and tumok (*Musa troglodytarum*) as compared to Vitamin E. Antioxidant potential of the extracts was tested and measured using *In Vitro* Lipid Peroxidation Assay as indicated by absorbance value in mAU and % Lipid Peroxidation Inhibition. Based on this test, all the fruits exhibited greater antioxidant potential than Vitamin E. Native passion fruit and rattan exhibited the greatest antioxidant potential and therefore, ranked 1.

KEYWORDS: absorbance value, antioxidant potential, indigenous fruits, *In Vitro* Lipid Peroxidation Assay, natural antioxidants

INTRODUCTION

Today, the important role of dietary antioxidants in maintaining integrity of living organisms is gaining recognition. Researches continue to grow regarding the importance of antioxidants as healthful components of food. Antioxidants are substances that delay or inhibit oxidative damage (Choy, Bezie, & Cho, 2000; Langseth, 1993). New data are constantly gathered to show the role of oxidative stress and the involvement of free radicals in the pathogenesis of degenerative diseases such as cancer and cardiovascular diseases (Vaya & Aviram, 2001; Khor, 2001). The most likely and practical way to fight degenerative diseases is to improve body antioxidant status which could be achieved by higher consumption of vegetables and fruits (Halvorsen et al., 2002; Miller et al., 2000; Ou et al., 2002). Studies have shown that increased consumption of fresh fruits and vegetables is associated with lower incidence of disease.

Recent studies have shown that protective mechanisms of fruits are thought to be attributed to the presence of natural antioxidants (Adom & Liu, 2002; Pellegrini et al., 2003). Studies on the antioxidant potential of common Philippine fruits such as bignay, kalumpit, tiesa, durian, bago (Philippine News Agency, 2009), katmon, rambutan, kamias, pomelo (Philippine Alternative Medicine, 2011), papaya ("Papaya," n.d.), guava (United States Department of Agriculture, 2007), mangosteen ("Mangosteen," n.d.), and noni ("Noni," n.d.) were reported. However, information on the chemical composition and antioxidant potential of indigenous fruits that are native and occurring naturally in the Cordillera region has not yet been done.

Therefore, identification of physiologically active constituents and investigation on the antioxidant potential of the following selected indigenous fruits namely ayosep (*Vaccinium angustifolium*), bitungol (*Flacourtia indica*), kalamondin (*Citrofortunella microcarpa*), marble tomato (*Lycopersicon pimpinellifolium*), native lacatan (*Musa paradisiaca*), native passion fruit (*Passiflora edulis*), pinit (*Rubus copelandii*), rattan (*Calamus mollis*), tamarillo (*Cyphomandra betacea*) and tumok (*Musa troglodytarum*) has been carried out.

MATERIALS AND METHODS

Phytochemical Screening of Fruits

The secondary metabolites such as alkaloids, steroids, anthraquinones, saponins, polyphenols, flavonoids, and tannins present in the edible portion of the fruits were determined using preliminary and confirmatory tests (Aguinaldo et al., 2004). The presence of alkaloids was detected using Mayer's and Dragendorff's tests and for the others the following were used: steroids using Keller-Killiani test for deoxysugars; Liebermann-Burchard test for unsaturated sterols and Kedde test for unsaturated lactones; anthraquinones using Borntrager's and modified Borntrager's tests; flavonoids such as leucoanthocyanins using Bate Smith and Metcalf test; flavonoids containing cyanidin-y-benzopyrene nucleus using Wilstatter "cyanidin" test; froth test for saponins while tannins and polyphenols using Gelatin and Ferric chloride tests; and, Guignard test for cyanogenic glycoside.

Preparation of Fruit Extracts and Control

Approximately 20 g of edible fruit portion including seeds or peelings was homogenized with 80 ml of 100% methanol (Tedia) in a blender (Kyowa KB 11096) for 1 min and was filtered through filter paper (No.1, Whatman Inc.). The residue was re-extracted using 20 mL methanol and then filtered. The extracts were combined and concentrated to a volume of 40 ml using a rotary evaporator (Heidolph) under partial vacuum at 80 °C (Mahattanatawee, 2006). The fruit extracts obtained through rotary evaporation were used in *In vitro* Lipid Peroxidation Assay. Liver homogenate with ferric chloride and commercially available Vitamin E (200 units) served as the positive control while liver homogenate treated with ferric chloride was the negative control.

Preparation of Homogenate

The liver of normal Wistar rat (*Rattus norvegicus*), an albino laboratory rat commonly used in biological and medical research was used in the study. After dissection, the specimen was perfused with ice cold 0.9% sodium chloride (NaCl) or Normal Saline Solution (NSS) to maintain normal cell turgidity and to avoid rapid oxidation. The tissue was homogenized at a concentration of 10 % w/v in 1.15% of potassium chloride (KCl) dissolved in distilled water using a mortar and pestle and was centrifuged at 3, 000 rpm for 10 minutes to obtain the rat liver homogenate.

In Vitro Inhibition of Lipid Peroxidation

Lipid peroxidation is a well established mechanism of cellular injury in both plants and animals. It is used as indicator of oxidative stress in cells and tissues (Oxford Biomedical Research, 2010; Oxis Research, 2010). The following steps were done in the study: Two drops (100 uL) of the fresh extract from the selected fruit were mixed with 0.5 ml of the homogenate. Lipid peroxidation was induced by adding two drops (100 uL) of 10% ferric chloride (FeCl3). Two drops (100 uL) of the commercially available 200 IU α tocopherol Vitamin E (Squibb) capsule, 0.5 ml homogenate and 2 drops FeCl3 solution were used as a standard (positive control) while the negative control was composed of 0.5 mL liver homogenate and 2 drops (100 uL) 10% ferric chloride only. The mixtures were incubated at 37 deg. C for 20 minutes and the reaction was stopped by adding 2 ml of ice cold 0.25N hydrochloric acid (HCl) containing 15% Tricholoroacetic acid, 0.38 % thiobarbituric acid and 0.05 % butylated hydroxy toluene. All tubes were heated at 80 deg. C for 15 minutes. Mixtures were centrifuged at 4,000 rpm for 5 minutes. Measurement of absorbance in milli-absorbance unit (mAU) of the supernatant was done using UV Vis spectrophotometer Dual Beam 550 (Labomed) at 532 nm. Calculation of % Inhibition of Lipid Peroxidation using the formula: % Inhibition = [(AB – AA)/ AB] x 100 where: AB- absorption of negative control sample; AA – absorption of tested extract solution (Rathee et al., 2006; Souri et al., 2008; Sundararajan et al., 2006).

Statistical Analysis

Quantitative data on absorbance values of the fruits and controls measured using *In vitro* Lipid Peroxidation assay in five replicates were treated statistically using one way Analysis of Variance (ANOVA). The 0.05 level of significance was used in testing the significance between two means. To locate where the difference lies, a follow up test, Tukey method was used. Finally, ranking was done to determine which among the fruits had the highest and lowest antioxidant potential.

RESULTS AND DISCUSSION

Phytochemical Constituents of the Fruits

Results (Table 1) show the presence of alkaloids in ayosep, native passion fruit, kalamondin, marble tomato, and tamarillo. According to Herraiz and Galisteo (2003), alkaloids such as aromatic β carboline alkaloids are present in fruits such as in raisins, grape, grape juice and wine. In the study they conducted using 2′2′- azino-bis or ABTS assay, alkaloids acted as antioxidants and free radical scavengers when absorbed and accumulated in the body. Steroids are present in all the fruits studied except lacatan and tumok. Steroids possess antioxidant properties. Plant phytosterols, such as β sitosterol found in peanuts, have anti-cancer health effects in several cases such as stomach, colon, breast and prostate cancer, and help lower LDL cholesterol level by blocking its absorption (Awad & Fink, 2000; Moriusi, 2006).

Saponins are present in all the fruits except ayosep, bitungol and tumok. Saponins work as antioxidants by inhibiting formation of free

Table 1.

Phytochemical Analysis Data of the Fruit Extracts

Family	Fruit Species	A	S	N	P	O	T	F	C
Arecaceae	Rattan (*C. mollis*)	-	+	-	+	+	+	-	-
Ericaceae	Ayosep (*V. angustifolium*)	+	+	-	-	-	-	+	-
Flacourtiaceae	Bitungol (*F. indica*)	-	+	-	-	+	+	+	-
Musaceae	Native lacatan (*M. paradisiaca*)	-	-	-	+	+	-	-	-
Musaceae	Tumok (*M. troglodytarum*)	-	-	-	-	+	-	-	-
Passifloraceae	Native passion (*P.edulis*)	+	+	-	+	+	-	-	-
Rosaceae	Pinit (*R. copelandii*)	-	+	-	+	+	-	+	-
Rutaceae	Kalamondin (*C. microcarpa*)	+	+	-	+	+	-	-	-
Solanaceae	Marble tomato (*L. pimpinellifollium*)	+	+	-	+	-	-	-	-
Solanaceae	Tamarillo (*C. betacea*)	+	+	-	+	-	-	+	-
Arecaceae	Rattan (*C. mollis*)	-	+	-	+	+	+	-	-
Ericaceae	Ayosep (*V. angustifolium*)	+	+	-	-	-	-	+	-
Flacourtiaceae	Bitungol (*F. indica*)	-	+	-	-	+	+	+	-
Musaceae	Native lacatan (*M. paradisiaca*)	-	-	-	+	+	-	-	-
Musaceae	Tumok (*M. troglodytarum*)	-	-	-	-	+	-	-	-
Passifloraceae	Native passion (*P. edulis*)	+	+	-	+	+	-	-	-
Rosaceae	Pinit (*R. copelandii*)	-	+	-	+	+	-	+	-
Rutaceae	Kalamondin (*C. microcarpa*)	+	+	-	+	+	-	-	-
Solanaceae	Marble tomato (*L. pimpinellifollium*)	+	+	-	+	-	-	-	-
Solanaceae	Tamarillo (*C. betacea*)	+	+	-	+	-	-	+	-

LEGEND: A– Alkaloids; S– Steroids; N– Anthraquinones; P– Saponins; O- Polyphenols; T– Tannins; F– Flavonoids; and C– Cyanogenic glycosides (+ positive, - negative) radicals (Balandrin, 2003). Polyphenols are present in all the fruits except marble tomato. Tannins are present only in rattan and bitungol. Tannins and polyphenols are powerful free radical scavengers and antioxidants (Mayer, 2005). The antioxidant activity of polyphenols are mainly due to their redox properties which play an important role in adsorbing and neutralizing free radicals, quenching oxygen or decomposing peroxides (Karou et al., 2005). Phenolic compounds such as caffeic, ferulic and gallic acid exhibit high free radical scavenging activities (Chen & Yen, 2007; Manach et al., 2004; Naczk & Shahidi, 2004).

Flavonoids are present only in ayosep, bitungol, pinit, and tamarillo. Flavonoids such as quercetin and kaempferol in tea, apples and berries possess antioxidative and radical scavenging activities (Bor et al., 2006; Pietta, 2000). Flavonoids such as quercetin in citrus fruits and ferulic acids in grains have been demonstrated to have anti-inflammatory, anti-allergenic, anti-aging and anticarcinogenic activity (Cody, 1986; Havsteen, 1983; Adom & Liu, 2002). Anthraquinones and cyanogenic glycosides are absent in all the fruits. Thus, the antioxidant potential of the fruits is not contributed by the said compounds.

Antioxidant Potential of the Fruits

The mean absorbance values and % lipid peroxidation inhibition of the various fruit extracts and controls in five replicates (Table 2) shows that all the fruits in this study possess antioxidant potential as indicated by their lower mean absorbance as compared to the positive control. A lower absorbance value indicates lesser amount of malondialdehyde formed as a byproduct of lipid peroxidation (McBride & Kraemer, 1999). It also implies higher % lipid peroxidation inhibition. Vitamin E and ferric chloride had an absorbance value of 0.8396 and 0.969 mAU. Native passion fruit has the lowest absorbance value in mAU (0.0942), and highest % lipid peroxidation inhibition (90.3%) followed by rattan (0.150) and ayosep (0.225). The fruit with the highest absorbance value (0.6396) and lowest % lipid peroxidation inhibition (34%) is marble tomato, followed by the two banana species, lacatan (0.5474) and tumok (0.4667).

Table 2.

Mean Absorbance Values (mAU) and % of Lipid Peroxidation Inhibition (LPI) of the Fruit Extracts and Controls

Family	Fruit Species	Mean (mAU)	% LPI	Rank
Passifloraceae	Native passion (*P. edulis*)	0.0942	90.3	1
Arecaceae	Rattan (*C. mollis*)	0.150	84.5	2
Ericaceae	Ayosep (*V. angustifolium*)	0.225	76.8	3
Rutaceae	Kalamondin (*C. microcarpa*)	0.253	73.9	4
Rosaceae	Pinit (*R. copelandii*)	0.259	73.3	5
Flacourtiaceae	Bitungol (*F. indica*)	0.3506	63.8	6
Solanaceae	Tamarillo (*C. betacea*)	0.439	54.7	7
Musaceae	Tumok (*M. troglodytarum*)	0.4667	51.8	8
Musaceae	Native lacatan (*M.paradisiaca*)	0.5474	43.5	9
Solanaceae	Marble tomato (*L. pimpinellifollium*)	0.6396	34	10
Positive control (Homogenate+ 10% Ferric chloride solution + Vitamin E)		0.8396	13.4	
Negative control (Homogenate + 10% Ferric chloride solution)		0.969	0	

To further analyze and interpret the data gathered, the absorbance values of the different fruit extracts and controls were subjected to statistical treatment, Analysis of Variance (ANOVA).

Table 3.

ANOVA for the Test of Significance of Absorbance Values Between Fruits and Control

Sources of Variations	Sum of Squares	DF	Mean Square	F-value	Tabular Value	Interpretation
Between Groups	4.0849	11	0.3714	520.684	1.9946	Significant
Within Groups	0.03423	48	0.000713			
Total	**4.1191**					

The data (Table 3) show that the computed F-value of 520.684 is greater than the tabular value of 1.9946 implying significant difference. This means that the antioxidant potential between and among the fruits also between fruits and controls greatly differ from each other. To determine where the significance lies, a follow-up test, the Tukey method, was employed.

Table 4.

Multiple Comparison Test of the Absorbance Values of Fruits and Controls Using Tukey method

FRUIT/ CONTROL	T	I	P	A	B	U	L	K	M	F	E
R	0.289	0.109	0.0558	0.075	0.201	0.317	0.397	0.103	0.490	0.819	0.690
T		0.179	0.3448	0.214	0.0884	0.028	0.108	0.186	0.201	0.530	0.401
I			0.165	0.0342	0.091	0.207	0.288	0.0062	0.380	0.710	0.580
P				0.131	0.256	0.372	0.453	0.159	0.545	0.875	0.745
A					0.126	0.242	0.322	0.028	0.415	0.744	0.615
B						0.116	0.197	0.0976	0.289	0.618	0.489
U							0.081	0.2136	0.173	0.502	0.373
L								0.2944	0.092	0.422	0.292
K									0.387	0.716	0.587
M										0.329	0.200
F											0.1294

NOTE: Honestly Significant Difference (HSD): 0.0582
[Mean difference (MD) > HSD value, significant; MD ≤ HSD value, not significant]

LEGEND: Rattan (R), Tamarillo (T), Pinit (I), Passion (P), Ayosep (A), Bitungol (B), Tumok (U), Lacatan (L), Kalamondin (K), Marble Tomato (M), Ferric chloride (F), and Vitamin E (E)

Mean difference is significant

Controls

Mean difference is not significant

Fruit samples

In showing the multiple comparisons of each selected fruit with other fruits using Tukey method (Table 4), if the mean difference in the absorbance value is higher than the HSD value of 0.0582, it implies that the difference is significant. Thus, it indicates that the antioxidant potential of the fruit is significantly different from the fruit to which it is compared. However, if the mean difference in the absorbance value is lower than or equal to the HSD value, it implies that the difference is not significant. Therefore, the antioxidant potential of the fruit is the same with the antioxidant potential of the fruit to which it is compared. The mean differences in the absorbance values of the fruits as compared to the negative and positive control are all higher than the HSD value implying that the difference is significant. This infers that all the fruits possess antioxidant potential higher than the controls. Along with these, the final ranking of the fruits (Table 5) based on their absorbance value indicating their antioxidant potential using Tukey method shows some important results.

Table 5.

Antioxidant Potential Ranking of the Fruits

Family	Fruits	Mean Absorbance Value (mAU)	A	S	P	O	T	F	Rank
Passifloraceae	Native Passion Fruit	0.0942	+	+	+	+	-	-	1
Arecaceae	Rattan	0.150	-	+	+	+	+	-	
Ericaceae	Ayosep	0.225	+	+	-	-	-	+	2
Rutaceae	Kalamondin	0.253	+	+	+	+	-	-	
Rosaceae	Pinit	0.259	-	+	+	+	-	+	
Flacourtiaceae	Bitungol	0.3506	-	+	-	+	+	+	3
Solanaceae	Tamarillo	0.439	+	+	+	-	-	+	4
Musaceae	Tumok	0.4667	-	-	-	+	-	-	
Musaceae	Native Lacatan	0.5474	-	-	+	+	-	-	5
Solanaceae	Marble Tomato	0.6396	+	+	+	-	-	-	6

LEGEND: A—Alkaloids; S—Steroids; P—Saponins; O—Polyphenols; T—Tannins; and F—Flavonoids (+ positive, - negative)

Native passion fruit and rattan are both ranked 1. Based on the phytochemical results, both contain four different phytochemicals. Steroids, saponins and polyphenols are present in both fruits while alkaloids are only present in passion fruit and tannins only in rattan. Each constituent exhibits antioxidant activity. The presence of these constituents may signify that both fruits contain phytochemicals that work together in a synergistic action to inhibit *in vitro* lipid peroxidation.

Chaudiere and Ferrari-lliu (1999) state that different antioxidant metabolites may have synergistic and interdependent effects on one another. In their study, antioxidants degraded peroxides and free radicals through co-operative interactions. Intracellular antioxidants such as ascorbate, glutathione, and carotenoids among others worked together to degrade superoxide and hydroperoxides. Ascorbate and glutathione scavenge oxidizing free radicals by means of hydrogen atom transfer while carotenoids are secondary scavengers of free radicals but physical quenchers of singlet oxygen. According to Adom and Liu (2002) and Svilaas et al. (2004), recent research has shown that the complex mixture of phytochemicals in food provides better protective health benefits than single phytochemicals through a combination of additive and or synergistic effects. Highest levels of antioxidants in the form of special phytochemicals called polyphenols and thiols (flavones, anthocyanins, ellagic acid, isoflavones, stilbenoids, etc.) present in fruits such as berries work together in synergistic ways (Vertuani, Angusti, & Manfredini, 2004). In this study, the combination of four phytochemicals in both passion fruit and rattan resulted in their highest antioxidant potential. Although alkaloids are absent in rattan, tannins are present which may equal the potential activity of alkaloids in native passion fruit (EPPA, 2002). Tannins and alkaloids possess antioxidant property. Both are active principles in scavenging nitric oxide (NO) (Bor et al., 2006).

Among all the fruits, marble tomato had the lowest antioxidant potential as indicated by its highest absorbance value. This may be due to the absence of polyphenolic compounds such as flavonoids that contribute strong antioxidant activity. Flavonoids act as strong superoxide and hydroxyl scavenger during lipid peroxidation (Middleton, Kandaswami, & Theoharides, 2000). Chelation of free iron is another property of flavonoids that contributes to preventing oxidative reactions (Mazza & Oomah, 2000). However, based on the phytochemical results, marble tomatoes contain alkaloids, phytosterols and saponins which possess antioxidant properties. The presence of these constituents did not yield a high antioxidant potential since according to Vertuani et al. (2004), some phytochemicals when present in fruits together with other compounds may exhibit poor reactivity and weak interaction with each other. Negative interactions (interferences) occur when certain components of the mixture inhibit the biological activity of pharmacologically active compounds by reducing their stability or bioavailability. In teas, proanthocyanidins interfere with caffeine. Mixture of plant flavonoids has shown inhibitory influence on ATPase enzymes (Lila & Raskin, 2005). In addition, marble tomatoes contain lycopene. Several studies have indicated that lycopene is an effective antioxidant and free radical scavenger (Chen et al., 2001; Rao & Agarwat, 2000). But there were reports that the contribution of phenolic compounds to antioxidant activity was much greater than Vitamin C and carotenoids such as lycopene (Mahattanatawee, 2006).Thus, carotenoids are not particularly good quenchers of peroxyl radicals relative to phenolics (Prior, Wu, & Schaich, 2005).

CONCLUSION

The presence of bioactive phytochemicals such as alkaloids, steroids, saponins, polyphenols, flavonoids and tannins are responsible for the antioxidant potential of the fruits. All the fruits possess greater antioxidant potential than Vitamin E (200 IU). The daily requirement for Vitamin E is 200 to 400 IU. Therefore, the fruits tested in this study are recommended to be eaten daily than intake of commercially available vitamin E. Among the ten fruits tested, native passion fruit (Passifloraceae) possesses the greatest antioxidant potential. Marble tomato (Solanaceae), on the other hand, possesses the lowest antioxidant potential followed by native lacatan and tumok (Musaceae). Fruits belonging to Arecaceae (rattan), Ericaceae (ayosep), Flacourtiaceae (bitungol), Passifloraceae (native passion fruit), Rosaceae (pinit), and Rutaceae (kalamondin) exhibit high antioxidant potential *in vitro*. The antioxidant potential between and among the fruits are significantly different except between rattan and native passion fruit and among ayosep, kalamondin and pinit where the antioxidant potential is equal between and among them respectively.

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