TOTAL FLAVONOIDS, TOTAL PHENOLICS AND VITAMIN C: INDICATORS OF THE ANTIOXIDANT POTENTIALS OF SELECTED FRUIT PEELS

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Abstract— The environment has been dumped with various solid wastes. A good part of this kind of wastes are the peels from fruits. Recent scientific researches have discovered the worth of the fruit peels in terms of substances that are good for the health. This study determined the antioxidant properties in terms of total flavonoids, total phenolics and vitamin C of the methanolic extract of the ten (10) selected fruit peels, namely, rambutan (*Nephelium lappaceum*), mango (*Mangifera indica*), marang (*Artocarpus odoratissimus*), santol (*Sandoricum koetjape*), soursop (*Annona muricata*), lakatan (*Musa sapientum*), avocado (*Persia americana*), lanzones (*Lansium domesticum*), watermelon (*Citrullus vulgaris*) and dragon fruit (*Hylocereus undatus*). Quantitative analysis showed that lanzones had the highest total flavonoids (213.45 mg quercetin/g dried material). Rambutan was high in both vitamin C (36.47 mg ascorbic acid/g dried material) and total phenolics concentration (96.69 mg GAE/g dried material). Dragon fruit came out with the lowest total flavonoids (5.70 mg quercetin/g dried material) and total phenolics (0.66 mg GAE/g dried material); no vitamin C was detected. Flavonoids, phenolics and vitamin C are recognized antioxidants. Analysis of the 10 selected fruit peels showed the total flavonoids content to have this order (highest to lowest concentration) – lanzones > santol > rambutan > marang > watermelon > mango > lakatan > avocado > soursop > dragon fruit. In terms of total phenolics, rambutan > mango > santol > marang > lanzones > soursop > lakatan > watermelon > avocado > dragon fruit. For vitamin C, rambutan > lanzones > lakatan > watermelon > soursop > marang > mango > avocado > santol > dragon fruit.

Keywords— Antioxidants, Total Flavonoids, Total phenolics, Vitamin C, Fruit peels.

I. INTRODUCTION

Fruit peels usually just end up as part of solid wastes. Being organic in nature these can be subjected to composting but still their volume keeps on increasing every day. Environmental problem of this kind can be lessened by utilizing the fruit peels instead of dumping them, thus, there is a need to study them to discover something valuable.

Majority of the health-promoting substances come from plants of all kinds. Aside from this benefit, plants are also responsible for taking up the carbon dioxide in the atmosphere. Considering the complicated environmental problems being experienced by man, cutting these intact plants, even for a righteous purpose, may worsen the problems instead of lessening them. Aside from global warming, increasing build-up of solid wastes is another major environmental problem in which fruit peels are one of the main contributors.

The nutritional value and antioxidant potential of many fruits, seeds and their rinds or peels have not been given much attention such that most often these parts of fruits are simply discarded. It is, therefore, necessary to evaluate the nutritional and antioxidant values of these fruit waste materials so that the knowledge derived can be used to encourage re-utilization of the seeds and rinds in possible value-added applications (Johnson et al., 2012). Interestingly, the seeds and rinds/peels of some fruits have higher vitamins, fibers, minerals and other essential nutrients than the pulp fractions (Jayaprakasha et al., 2001). Hopefully, many fruit wastes, especially the rinds and peels, will no longer be considered wastes because these can have great contribution in the field of health promotion. In the process, the efforts in this direction can help reduce the volume of solid wastes.

Among the different living organisms, plants are in a difficult situation since they cannot control their environment nor move away from stressful conditions that they cannot cope up with. Thus, different forms of defense are evolved by them (Del Rosario & Palmes, 2003). Beyond some forms of physical defense like growing thorns, plants elaborate a rich arsenal of chemical defense. These chemical defenses are secondary metabolites such as antifeedants (against herbivores), foul tasting and smelling compounds, toxins, and antioxidants (Timberlake, 2000).

Antioxidants have the ability of protecting organisms from damage caused by free radical-induced oxidative stress (Li, 1999). During oxidative stress and exposure to radiation, excessive free radicals are produced that are known to cause damage to the biomolecules (Halliwell and Gutteridge, 1993).

Antioxidant Properties

The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, coumarins, curcumins, lignin, saponin, plant sterol, and more (Lucia et al., 2008). It is an inherent property of most living organisms to produce substances such as hormones in humans that are necessary to regulate body processes. If human beings can produce antibodies to fight foreign microbes, plants as well have phytochemicals that are considered to be very powerful to combat diseases in humans and even preventing their occurrence.

Phenolics and flavonoids.
As stated by Goyal et al. (2010), the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. In this respect, polyphenolic compounds like flavonoids and phenolic acids commonly found in plants have been reported to have multiple biological effects, including an antioxidant activity.
Flavonoids are a group of polyphenolic compounds naturally present in most edible fruits and vegetable plants. They are secondary plant metabolites and are natural antioxidants which help to maintain the body's health and to protect against diseases. They also exhibit detoxifying properties due to their metal chelating capabilities. Epidemiological and clinical studies have provided evidence of a potential role for flavonoids in lowering the risk of coronary heart diseases prevention, cardiovascular diseases, Alzheimer's disease, neurodegenerative diseases, diabetes, osteoporosis, and lung cancer (Lampila *et al.* ,2009).
Vitamin C (L-ascorbic acid) is an essential cofactor for enzymatic reactions required for collagen hydroxylation, carnitine biosynthesis, and norepinephrine formation (Levine et al. 1999). Vitamin C also functions as a chemical reducing agent or antioxidant. Unlike most mammals, humans do not synthesize ascorbate de novo, and the vitamin must be ingested (Nishikimi et al. 1994).

II. DETAILS EXPERIMENTAL

2.1. Plant material
Ripe intact fruit samples, namely, rambutan (*Nephelium lappaceum*), mango (*Mangifera indica*), marang (*Artocarpus odoratissimus*), santol (*Sandoricum koetjape*), soursop (*Annona muricata*), lakatan (*Musa sapientum*), avocado (*Persia americana*), lanzones (*Lansium domesticum*), watermelon (*Citrullus vulgaris*) and dragon fruit (*Hylocereus undatus*) were purchased from the fruit stands (Fig.1) in Cagayan de Oro City in May 2014 . Each ripe fruit was washed using detergent with gentle brushing (Fig.2) under running tap water followed by rinsing with distilled water. The fruits were then allowed to drain, after which careful removal of peels was done (Fig.3). The peels were cut into small pieces using a clean knife. Drying of the plant materials in an air-conditioned laboratory was done for a period of 10 to 12 days. The dried peels were then subjected to further particle size reduction by milling using an electric mill.


Fig.1 Intact fruits


Fig.2 Thorough washing


Fig.3 Peeling

2.2. Preparation of Plant Extracts
Twenty (20) grams of dried powdered fruit peels were soaked with 300 mL of methanol for 12 hours with occasional shaking. This mixture was filtered and the filtrate was set aside. The residue was again soaked with another 300 mL of methanol for one hour and the filtrate was collected. The residue was soaked again with 300 mL methanol, and filtered. The filtrates were then combined and concentrated to about 200 mL using the rotary evaporator.

2.3. Crude Extract Concentration
Twenty (20) ml of extract solution was pipetted into a pre-weighed aluminum dish. This dish was placed in an oven to evaporate the methanol at a temperature three degrees (3^O) higher than the boiling point of methanol. Repeated oven drying and weighing were done until constant weight of the solids was achieved. The concentration of the crude extract was calculated by dividing the mass of the residue by the volume of sample (20 mL). A dilution of 1mg/mL was prepared for the subsequent tests.

2.4 Total Flavonoids
The total flavonoids of the fruit peel was determined with the use of aluminum chloride ($AlCl_3$) method using quercetin as the standard. One (1) mL of the methanolic extract was diluted to 10 mL solution. One (1) mL of diluted solution was added with 5 mL distilled water followed by addition of 300 µL of 5% $NaNO_2$. The mixture was incubated at room temperature for 5 minutes. After the incubation period, about 0.60 mL of $AlCl_3$ solution was added. Further incubation was done for a period of 6 minutes to allow the chemical reaction to take place completely. It was then treated with 2 mL of 1 mmole NaOH then diluted with 1.1 mL of distilled water and incubated further for 20 minutes at room temperature. The absorbance was read at 510 nm using UV-Vis spectrophotometer. The total flavonoids was then calculated from a quercetin standard curve. Triplicates were made for each fruit peel sample.
2.5 Total Phenolics.
The total phenolics of the fruit peel was determined using the Folin-Ciocalteu (FC) reagent method with

slight modification. A volume of 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 minutes. Gallic acid was used as the standard. The total phenolics, expressed as milligrams gallic acid equivalent (GAE) per gram of dried material, was determined from the prepared calibration curved. Triplicates were made for each fruit peel sample.

2.6 Vitamin C

The vitamin C content of the aqueous extract was determined using the method demonstrated by Benderitter et al.,1998. Briefly, 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO4.5H_2O$ in 100mL of 5M H2SO4) was added to 500 µL reaction mixture of 300 µL appropriate dilution of hydrophilic extract with 100 µL of 13.3% trichloroacetic acid and distilled water. The reaction mixture subsequently incubated for 3 hours at 37^0C, then 0.5mL of 65% $H2SO_4$ (v/v) was added to the medium, and the absorbance was measured at 520 nm, and the vitamin C content of the sample was then subsequently calculated from the calibration curve prepared with ascorbic acid as standard.

III. RESULTS AND DISCUSSION

3.1 Total Flavonoids

As shown in Table 1, among the 10 fruit peels, lanzones has the highest concentration (213.45 mg quercetin/g dried material) of total flavonoids which is very much higher than those of santol and rambutan having total flavonoid concentrations of only 55.97 and 51.24, respectively. Dragon fruit showed the lowest concentration with only 5.71.

Table 1. Total flavonoids, total phenolics and vitamin C concentration of the ten selected fruit peels.

Fruit peels	Total flavonoids	Total phenolics	Vitamin C
Avocado	13.70	1.70	2.03
Dragon fruit	5.71	0.66	-0.85
Lakatan	18.60	4.24	17.60
Lanzones	213.45	9.42	19.65
Mango	18.60	56.12	4.60
Marang	25.44	17.77	5.81
Rambutan	51.24	96.69	36.48
Santol	55.97	23.53	1.92
Soursop	12.12	6.64	8.05
Watermelon	23.27	2.01	12.43

Total flavonoids expressed in mg quercetin/g dried material
Total phenolics expressed in mg GAE/g dried material
Vitamin C expressed in mg ascorbic acid/g dried material

3.2. Total Phenolics

The fruit peels of rambutan contain the highest total phenolics ((96.69 mg GAE/g dried material) followed by mango (56.12) then santol (23.53) and marang (17.77). Lanzones ranks fifth with 9.42, this is unlike its total flavonoids having the highest concentration among the ten fruit peels.

The antioxidant activity of phenolics is mainly because of their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans, 1995).

3.3 Vitamin C

The vitamin C concentration shown in Table 1 indicates that rambutan peels are high in vitamin C with 36.48 mg ascorbic acid/g dried material while lanzones has 19.65, a difference of almost half of that of rambutan. Lakatan and watermelon have concentrations 17.60 and 12.43, respectively. Peels of santol contain the lowest vitamin C concentration (1.92), and nothing was found in dragon fruit peels.

Figure 4 summarizes all the data in a bar chart. It shows that rambutan is consistently high in total phenolics and vitamin C concentration. This is interesting because, so far, rambutan peels have not been known to have been utilized for any health-promoting purposes.

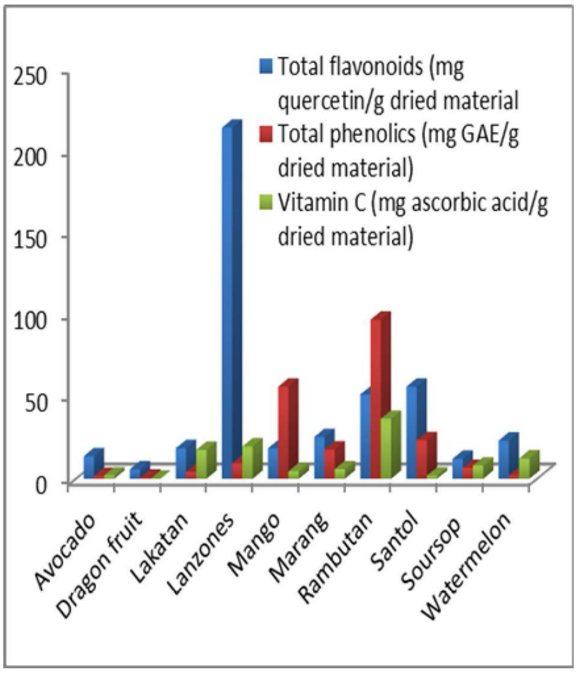

Fig.4. Comparison of the total flavonoids, total phenolics and Vitamin C concentration of the selected fruit peels.

CONCLUSIONS

With the use of accepted methods, this study has shown that the peels of the commonly eaten fruits are potential sources of antioxidants. Of the ten selected fruit peels, rambutan has high contents of total phenolic compounds and vitamin C while lanzones is very high in total flavonoids. Therefore, utilizing these peels as sources of antioxidants is more beneficial instead of dumping them as solid wastes.

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