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Antioxidant Property and Total Polyphenol and Flavonoid Content of Selected Fruits and Fruit Wines

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Abstract

The fruits *Vaccinium myrtoides* ('ayosep'), *Annona muricata* ('guyabano'), *Garcinia mangostana* (mangosteen), *Ananas comosus* (pineapple), and *Fragaria × ananassa* (strawberry) are consumed either raw or as wine products. Given the paucity of data on the phenolic content of these fruits and their wine products, this study has quantified and compared their total polyphenol and flavonoid content using Folin-Ciocalteu and aluminium chloride method. The fruits and their wine derivatives were also evaluated for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl assay compared to Vitamin E (positive control). Results show that 'ayosep' contains the highest polyphenol (59.12 ± 0.0006 mg gallic acid equivalent per 100g fresh weight) and flavonoid (1186.44 ± 0.21mg quercetin equivalent per 100g fresh weight). Among all the fruits and wines, strawberry fruit and pineapple wine have the highest and lowest 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity with 92.35% (± 0.69) and 9.94% (± 8.58), respectively. Post-hoc analyses of results using Tukey test show that all the fruits and wines have equal activity with Vitamin E except pineapple wine. Fresh fruits particularly strawberry, mangosteen, 'guyabano' and 'ayosep' are recommended as sources of natural antioxidants and as alternatives to Vitamin E.

Keywords: antioxidant property, Benguet province, DPPH radical scavenging activity, flavonoid, fruits, total polyphenol, wines

Introduction

Fruits are known to possess beneficial secondary metabolites that promote health (Lizardo, Mabesa, Dizon, & Aquino, 2014). Studies show that the consumption of fruits rich in polyphenols and flavonoids increases protection against degenerative diseases such as cardiovascular problems and diabetes (Fredes, Montenegro, Zoffoli, Santander, & Robert, 2014; Nadja, Dyduch-Sieminska, Dyduch, & Gantner, 2014). Fruit wines are also reported to contain polyphenols and flavonoids, and the effects of these compounds in preventing the incidence of cancer have also been studied (Romagnolo & Selmin, 2012). As a result, moderate wine drinking is widely promoted to reduce the risks for cancer and heart diseases (IARC, 2010).

In the Philippines, locally available and commonly consumed fruits are processed into wines for home consumption or commercial purposes. Unfortunately, there is a dearth of literature reporting on the phenolic content and antioxidant activities of local fruits and their wine derivatives (of the few studies, see Belina-Aldemita, Sabularse, Dizon, Hurtada, & Torio, 2013). These data could otherwise be used by farmers, wine makers, and entrepreneurs in marketing their products. In Benguet province, fruits like *Vaccinium myrtoides* Miq. ('ayosep'), *Garcinia mangostana* Linn. (mangosteen), *Ananas comosus* Linn. (pineapple), *Annona* *muricata* Linn. (‘guyabano’), and *Fragaria* × *ananassa* Linn. (strawberry) are sold as raw fruits and homemade wines in the local markets. However, to date no literature is reported on these fruits and their wine derivatives.

Aiming to fill in this gap, this study has quantified and compared the total phenolic content (polyphenol/flavonoid) of the abovementioned fruits and their homemade wines in order to evaluate their quality and determine which ones are good for consumption. In addition, the study looked into the antioxidant activity of the fruits and their wines. Fruit wine production requires fermentation that involves changes in structural integrity of the material, and this has both negative and positive effects on its antioxidant activity (Kavitha & Kuna, 2014). Hence, this study also compared the antioxidant activity of the fruits and their wine derivatives. Findings from this study can be very informative to consumers but especially to local wine manufacturers and entrepreneurs. These can be used in the promotion of locally available fruits and their homemade wines over imported fruits and fruit wines that have penetrated the local markets.

Materials and Methods

Collection, Transport and Storage of Raw Fruits and Fruit Wines

Fresh ripe fruits (1/4 to 1 kg) for each fruit species and three bottles each of their commercial wine derivatives (750 mL total volume, 12% alcohol content, and six month old) were purchased from the public markets in Baguio and La Trinidad (Figure 1 & 2). Fruits were collected by hand picking from June to August 2015. Random sampling was carried out in sufficient quantity. Fresh fruits were packed into an ice box and delivered to the Natural Sciences Research Unit of Saint Louis University in Baguio City. The fruits were washed initially with running tap water to remove dirt and other debris then with distilled water. Fruit samples were stored at -20 °C using an ultra low freezer

Figure 1. Fresh fruit samples: 1 *Ananas comosus* (pineapple), 2 *Annona muricata* (guyabano), 3 *Fragaria* × *ananassa* (strawberry), 4 *Garcinia mangostana* (mangosteen), 5 *Vaccinium myrtoides* (ayosep).

Figure 2. Bottled fruit wine samples:
1 Ananas comosus *(pineapple),* 2 Annona muricata *(guyabano),* 3 Fragaria × ananassa *(strawberry),* 4 Garcinia mangostana *(mangosteen),* 5 Vaccinium myrtoides *(ayosep).*

(Legaci, USA). The wine bottles were kept at 5-8 °C in the dark until analysis.

Preparation of Raw Fruit and Fruit Wine Extracts

The fresh edible portion of each fruit (20 g) was used for the preparation of the crude fruit extract according to the methodology of Rawat, Jugran, Giri, Bhatt, and Rawal (2011). Parts were homogenized in 50 mL (80% v/v) methanol (Merck, Germany) using a blender (Imarflex 3216GC, Japan) for five minutes and were soaked for 24 hours. Extracts were filtered using Whatman No. 1 (Sigma Aldrich, United Kingdom) filter paper, and filtrates were centrifuged using an automated centrifuge (Labofuge 200, Germany) at 5300 rpm for 10 minutes. Supernatants were stored at 4°C prior to use within two days. As for the fruit wines, the samples were used directly from their bottles, following Stratil et al. (2008) and Tarko, Duda-Chodak, Sroka, Satora and Jurasz (2008). However, centrifugation and filtration of the fruit wines were also performed as previously described to remove solid particles or dense solid mass in the liquid. All extractions were performed in triplicate.

Determination of Total Polyphenols in Raw Fruits and Fruit Wines using Folin-Ciocalteu Assay

Two hundred fifty (250) µL of extract (raw fruit or fruit wine), 2,250 µL distilled water, and 250 µL Folin-Ciocalteu reagent (Merck, Germany) were mixed and allowed to stand for reaction up to five minutes. This mixture was neutralized by 2,500 µL of 7% sodium carbonate (w/v) and was kept in the dark at room temperature for 90 minutes. The absorbance of resulting blue color was measured at 765 nm using VIS spectrophotometer (Apel PD 303, Japan). Quantification was done on the basis of standard curve of gallic acid (Merck, Germany) prepared in 80% methanol. The results were expressed in milligrams gallic acid equivalent (GAE) per 100 grams fresh weight (fw) of the raw fruits and GAE/100 mL for the fruit wines (Rawat et al., 2011). Fresh weight was used because the fruits studied are commonly consumed raw or fresh. The standard curve was prepared using 0, 5, 10, 15, 20, 25 mg/ 100 mL solutions of gallic acid in methanol: water (50:50 v/v) (Ghasemi, Ghasemi, & Ebrahimzadeh, 2009). All values are expressed as the mean for three replications.

Determination of Total Flavonoids in Raw Fruits and Fruit Wines using Aluminum Chloride Method

A 500 µL solution of each extract (raw fruit or fruit wine) was mixed separately with 1500 µL of methanol, 100 µL of 10% aluminum chloride (Ajax, Australia), 100 µL of 1 M potassium acetate (Calbiochem, San Diego CA), and 2,800 µL of distilled deionized water. After mixing, the tubes were left in the dark at room temperature for 30 minutes. The absorbance of the reaction mixtures was measured at 415 nm with a VIS spectrophotometer (Apel PD 303, Japan). The calibration curve was prepared by using quercetin solutions (Calbiochem, San Diego CA) prepared in 80% methanol at concentrations 1, 250 mg to 10,000 mg/100 mL in methanol based on the methods of Adedayo, Oboh and Akindahunsi (2010). The flavonoid content was expressed as the mean (milligrams of quercetin equivalents (QE) per 100 g of fresh weight of the raw fruit and QE per 100 mL of the fruit wine) ± SD for three replications.

Antioxidant Activity Screening using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

A 50 µL sample extract (fruit and fruit wine), was mixed with 2 ml of DPPH (24 mg/L) solution in 80% methanol prepared in aluminum foil-wrapped test tube. The reaction mixture was shaken thoroughly for one minute using a vortex mixer and was left in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against a blank (methanol) using a VIS spectrophotometer. The % inhibition of DPPH radical caused by the raw fruit or fruit wine sample was calculated using the equation: DPPH radical scavenging activity (%)= $\{[(Abs_{neg\ control} - Abs_{sample})]/ (Abs_{neg\ control})\}x\ 100$ where $Abs_{neg\ control}$ is the absorbance of DPPH radical and methanol; Abs_{sample} is the absorbance of DPPH radical plus raw fruit extract/fruit wine extract/ control based on Du, He, Shi, Li, Li, and Zhu (2012). Experiments were carried out in triplicate and results are expressed here as mean values.

Statistical Analysis

Evaluation and analysis of data were performed using SPSS 20.0 for Windows software package. There was a minimum of three repetitions for the whole analysis. A single-factor Analysis of Variance (ANOVA) test with a post hoc Tukey test was applied to assess the differences between means. The quantitative data on the total polyphenol content (mg GAE/100 grams fresh weight for raw fruits and mg GAE/ 100 mL for fruit wine) and the total flavonoid content (mg QE/100 grams fresh weight for raw fruits and mg QE/ 100 mL for fruit wine) were measured and ranked using Folin Ciocalteu assay and aluminum chloride method, respectively.

Results and Discussion

Total Polyphenol and Flavonoid Content of Raw Fruits and Fruits Wines

All the fruits and wines studied contain polyphenols. *V. myrtoides* ('ayosep') fruit has the highest polyphenol content of 59.12 (± 0.0006) mg GAE per 100g fw, followed by *F. ananassa* (strawberry) fruit with 58. 44 (± 0.01) mg GAE/ 100g fw (Figure 3). This confirms earlier findings that berries such as *Vaccinium* and *Fragaria* spp. contain a high concentration of polyphenols (Pineli, Moretti, Santos, Campos, Brasileiro, Cordova, & Chiarello, 2011). Of all fruits, *A. comosus* (pineapple) has the lowest polyphenol content (40.03 ± 0.04 mg GAE/100g fw).

Among all the fruit wines, *G. mangostana* (mangosteen) has the highest polyphenol content of 23.94 (± 0) mg GAE per 100 mL, followed by *A. muricata* ('guyabano') wine with 23.18 (± 0.01) mg GAE/100 mL. Meanwhile, *A. comosus* (pineapple) wine has the lowest polyphenol content of 11.04 (± 0) mg GAE/100 mL.

It was observed that the different fruit samples had higher polyphenol content as compared to the fruit wines. This could be due to wine aging in the bottles and fermentation that decrease the polyphenol content of fruit wines (Ivanova, Vojnoski, & Stefova, 2012). Moreover, clarification as part of the wine making procedures decreases their polyphenol content (Ritter, Maier, Schoplein, & Dietrich, 1992).

Flavonoids are commonly found in the edible pulp of fruits. Among the fruits, *V. myrtoides* ('ayosep') fruit has the highest flavonoid content of 1186.44 (± 0.21) mg QE/100g fw (Figure 4). *Vaccinium* species are one of the richest sources of flavonoids such as anthocyanins (Wang, Chen, & Wang, 2009). On the other hand, *A. muricata* ('guyabano') fruit had the lowest flavonoid content of 7.06 (± 0.01) mg QE/100g fw followed by *A. comosus* (pineapple) fruit (14.12 ± 0.01 mg QE/ 100g fw). Similar results were obtained in the study conducted by Onyechi, Nkiruka Ibeanu, Eme, and Kelechi (2012) where *A. muricata* was shown to contain 9.32 and 5.24 mg flavonoids per 100g fruit. Honey pineapple was also reported by Alothman, Bhat, and Karim (2009) to have low flavonoid content (1.24-4.14 catechin equivalents/ 100g fw).

Among wines, the highest flavonoid content was recorded in *V. myrtoides* (358.8 ± 0.6 mg QE/100 mL), and the lowest in *A. comosus* (22.6 ± 0.06 mg QE/100 mL). Data revealed that

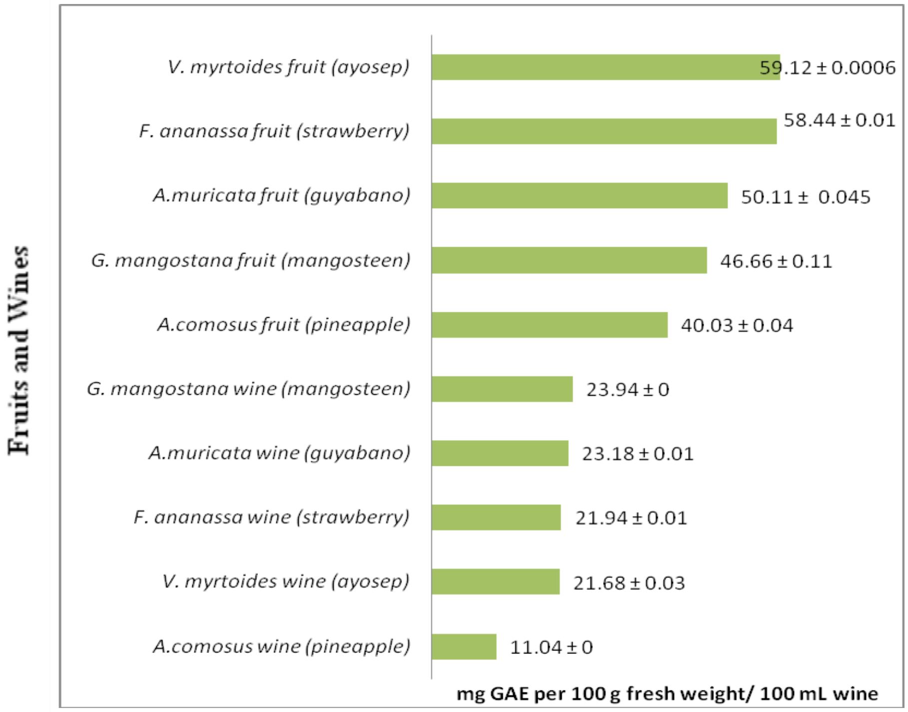

Figure 3. Total Polyphenol Content of Fruits and Wines

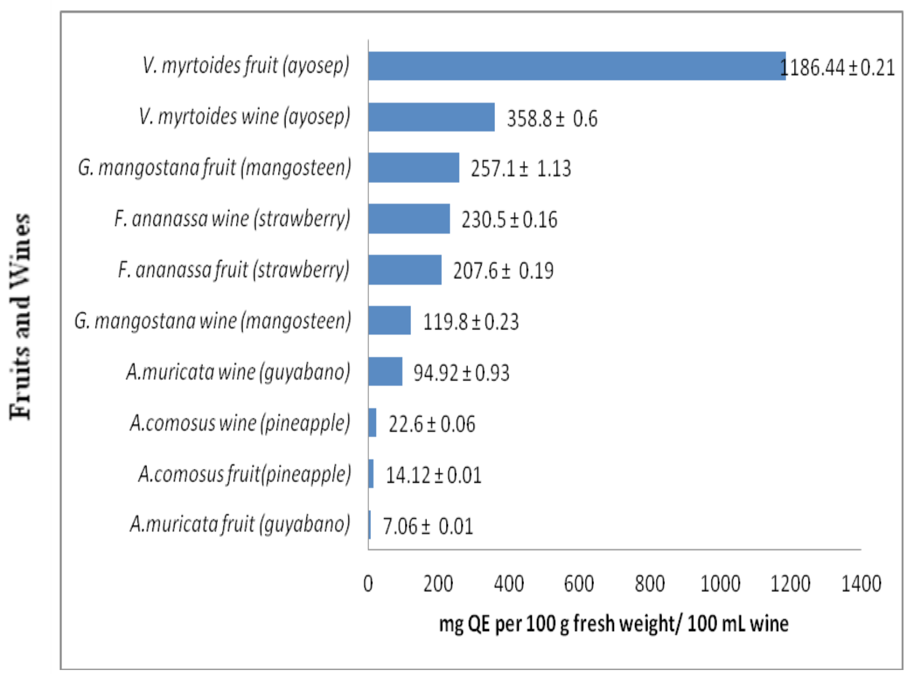

Figure 4. Total Flavonoid Content of Fruits and Wines

some wines contain more flavonoids than the fruits as in the case of strawberry, 'guyabano' and pineapple. Fermentation and alcohol content of the wines may have influenced the flavonoid content in the wines used in this study. Soleas, Diamandis, and Goldberg (1997) cited that fermentation can increase the amount of flavonoids present in the wines. In addition, ethanol produced during fermentation can act as a solvent in the extraction of pigments and tannins, thereby increasing the flavonoid content.

Antioxidant Activity of Fruits and Fruit Wines Using DPPH Assay

As shown in Figure 5, *F. ananassa* (strawberry) fruit exhibited the highest antioxidant activity as indicated by its 92.35% (±0.69) DPPH radical scavenging activity, followed by *G. mangostana* (mangosteen) with 89.08% (±8.02). This confirms earlier reports that strawberries are rich in antioxidants (Amini, Irian, Majd, & Mehrabian, 2013). Data gathered in this study is similar to those of Bursac Kovacevic, Levaj, and Dragovic-Uzelac (2009), where the antioxidant activity of fresh strawberry fruits is between 90.74% to 92.41% using DPPH radical scavenging assay. Sze Lim, Sze Hui Lee, and Chin Tan (2013) also confirm the antioxidant activity of *G. mangostana* pulp (24 ± 4 uM trolox equivalent g^{-1} dry weight) and pericarp (122 ± 2 uM TE g^{-1} dw).

A. comosus (pineapple) fruit was observed to have the lowest antioxidant activity (62.39 ±12.3 %). Almeida et al. (2011) also reported a low antioxidant activity of pineapple fruit using DPPH and ABTS assays expressed as Vitamin C equivalent antioxidant capacity (mg/100g) with 16.59 ± 0.86 and 58.59 ± 3.00, respectively.

Among the wines, *A. muricata* ('guyabano') and *A. comosus* (pineapple) has the highest (90.38± 2.51%) and lowest (9.94± 8.58%) antioxidant activity, respectively.

All of the fruits and fruit wines except *A. comosus* wine exhibited a significant DPPH

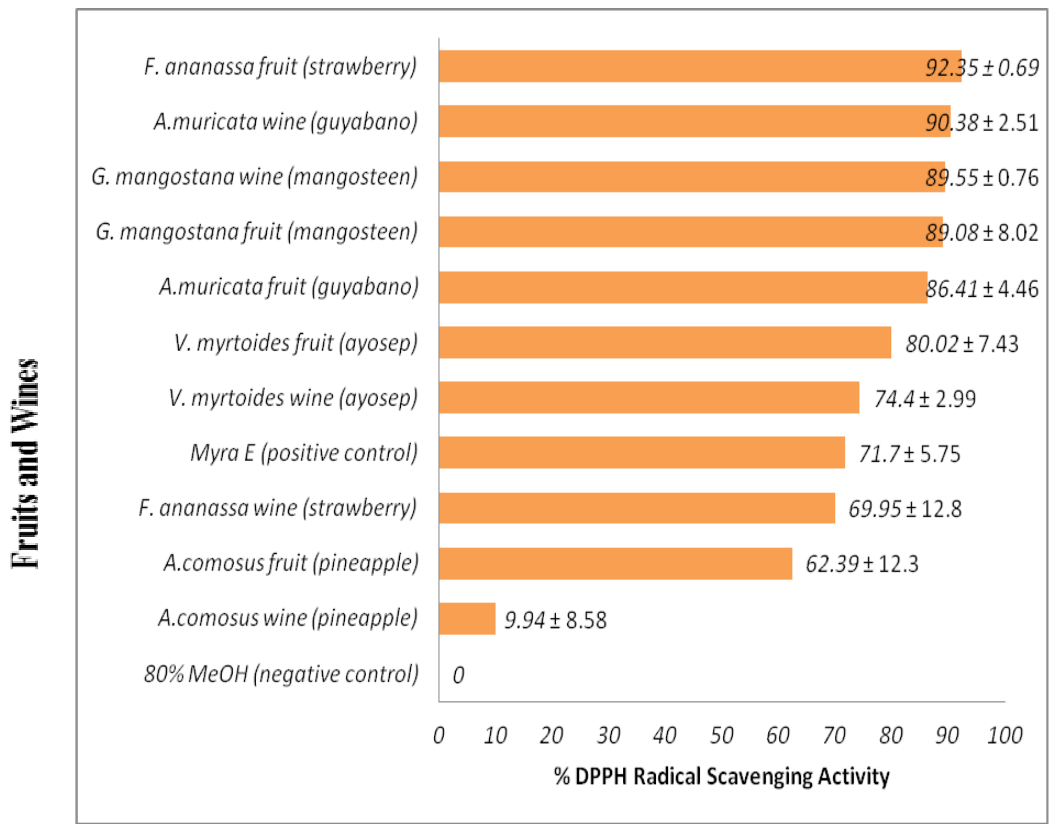

Figure 5. DPPH radical scavenging activity of the fruits, wines and controls.

radical scavenging activity (9.94 ± 8.58 %) as compared to the control, Vitamin E ($\rho<0.05$). Post hoc analysis of results using Tukey test revealed that all the fruits and wines, except pineapple wine, have equal antioxidant activity with Vitamin E. Based on the results, the wines of *A. muricata and G. mangostana* have higher antioxidant activity than their raw fruits. Wines may exhibit higher antioxidant activity because as Tarko et al. (2008) observed, the concentration of antioxidant compounds may increase twice and the level of antioxidants (flavonoids, catechins, resveratrol) can go up to 8 times higher due to the process of fermentation.

Conclusion

The study quantified and compared the total phenolic content and anti-oxidant activity of five local fruits and their homemade wines. All the fruits and wines contained polyphenols and flavonoid, with 'ayosep' fruit being the richest source for both antioxidants. Pineapple fruit had the lowest polyphenol content among all fruits, while 'guyabano' fruit had the lowest flavonoid content. Among wines, the highest flavonoid content was recorded in 'ayosep' wine and the lowest in pineapple wine.

Comparing the fruits and the fruit wines as groups, the fruit samples had higher polyphenol content than their fruit wine counterparts. This could be due to wine aging and clarification that tend to decrease the polyphenol content of fruit wines. On the contrary, some fruit wines posted higher flavonoids than their fruit counterparts as in the case of strawberry, 'guyabano' and pineapple. This may have been a result of alcohol production during fermentation, which tends to increase flavonoid content.

For anti-oxidant activity, strawberry fruit exhibited the highest, and pineapple fruit the lowest. Among wines, 'guyabano' and pineapple have the highest and lowest antioxidant activity, respectively. Overall, the fruits and fruit wines, except pineapple wine, have equal antioxidant activity with Vitamin E.

Although limited to five fruits and their wines, this study provides seminal basis for determining the best fruits and wines in Benguet province with regards to their phenolic content and antioxidant activity. Studies on other fruits and their wines should be conducted to provide more comprehensive data.

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