INTRODUCTION

Plant phenolics have potential applications in improving the quality of food. The control of the food deterioration due to oxidation of lipids, which leads to the development of off-flavor or odor, is a challenge faced by the food industry. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used to control the...
autoxidation of lipids and prolong the shelf-life of food products. However, the concerns about the safety of these synthetic substances have been raised, although there have been conflicting reports about the positive and negative effects of these substances on the human and animal health [Botterweck et al., 2000]. Nevertheless, the concerns on the toxicity of these synthetic phenoisols have justified the search of natural antioxidants from edible sources. The antioxidant properties of plant phenolics are well known [Kähkönen et al., 1999], making them good candidates as an alternative to the synthetic antioxidants currently used in the food industry.

The processing of fruit and vegetable generates large volume of wastes that can be sources of natural antioxidants. Phenolics are the most abundant secondary metabolites in plants and found in all plant organs [Dai and Mumper, 2010]. Thus, phenolics are present even in the peels and other nonedible parts of fruits and vegetables that ended up as wastes [Dimitrios, 2006]. A review of the worldwide waste generation reports showed that in 2010 the residues from the fruit and vegetable processing industry amounted to as low as 3.5M metric tons for grapefruit to as high as 130M metric tons for potatoes [Van Dyk et al., 2013]. The same set of data showed that fruit and vegetable processing resulted in losses due to wastes ranging from 3 to 7% in tomatoes to 40 to 80% in pineapple. Waste valorization, which is the production of useful chemicals, materials or energy from wastes, is an attractive management option for food processing residues [Arancon et al., 2013]. The recovery of antioxidants from fruit and vegetable wastes has already been demonstrated [Arancon et al., 2013; Federici et al., 2009].

Food grade solvents are expensive and often inaccessible to home-based or small-scale food processing facilities. Laboratory grade solvents may not be fit for human consumption and extraction of natural products using these solvents requires further purification steps if the extracts are meant to be added to food. The use of extracting media from edible sources will enable the direct application of the extracts to food. Alcoholic beverages have traditionally been used in extracting medicinal substances from plant sources [Akinsulire et al., 2007; Egea et al., 2015]. However, the use of alcoholic beverages in the extraction of phenolic substances from plants has not been extensively explored yet.

Distilled beverages are produced from distilling ethanol after the fermentation process and contain high amount of ethanol (about 40%). Neutral spirits, such as gin and vodka, are clear and colorless distilled beverages the flavor of which comes mainly from their alcohol content [Pauley and Maskell, 2017]. The level of phenolics in these spirits was too low to be measured [Goldberg et al., 1999] while the total antioxidant content was reported to be zero [Yashin et al., 2010].

This study was therefore designed to find out if neutral spirits could extract phenolics from the fruit and vegetable peels and whether the extracts possess the antioxidant activity.

MATERIALS AND METHODS

Materials and Reagents

The fruit and vegetable peelings were collected from local markets in the Science City of Muñoz, Nueva Ecija, Philippines: ripe banana (Musa acuminata), ripe mango (Mangifera indica), calamansi (Citrofortunella microcarpa), pineapple (Ananas comosus), squash (Cucurbita maxima), purple yam (Dioscorea alata), and sweet potato (Ipomoea batatas). The following reagent-grade chemicals were utilized: ethanol and methanol from Sharlau (Spain); 2,2-diphenyl-1-picrylhydrazyl (DPPH) and butylated hydroxianisole (BHA) from Sigma-Aldrich (Switzerland); gallic acid and ascorbic acid from Himedia Laboratories (India); sodium carbonate and Folin-Ciocalteau phenol reagent from Techno Pharmachem (India). The following distilled spirits were used: gin (locally manufactured, 80 proof), vodka (imported, 80 proof), and tequila flavored spirit (TFS, locally manufactured, 80 proof).

Extraction

The plant samples were cleaned, washed, cut into small pieces, air-dried and ground. The ground fruit and vegetable peel samples were separately extracted with 40% ethanol, gin, vodka or TFS (1 g plant material: 10 mL solvent) at room temperature for 48 hours. The extract was separated from the residue by filtration. The crude extract was collected and then placed in glass bottles that were protected from light and stored in the refrigerator until ready for analysis.
Determination of Total Phenolic using Folin-Ciocalteau Reagent

The method used by Musa and Abdullah [2011] for the determination of total phenolic content (TPC) was applied with a slight modification. A total of four hundred microliter (400 μL) of extract was added with 800 μL of distilled water and 1.0 mL diluted Folin-Ciocalteau phenol reagent (1:10). The solution was set aside for 5 minutes and then added with 1.0 mL of 7.5% (w/v) sodium carbonate. A series of standards of gallic acid was prepared. The absorbance of the extracts and the standard solutions at 765 nm were read using Spectrophotometer 1500 (Unico, USA). The total phenolic content (TPC) in the plant extract was expressed in milligram of gallic acid equivalent per gram dry weight of sample (mg GAE/DW).

Determination of Antioxidant Activity using DPPH Radical Scavenging Method

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay described by Chan, Lim, and Omar [2007] was performed with minor modification. Different solutions of the extracts (1000 ppm, 100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm) were prepared, while the DPPH solution was made by dissolving 6.0 mg of DPPH in 100 mL methanol. Exactly 1.5 mL of extract was added into the test tube containing 2.5 mL of DPPH solution. BHA served as standard. The mixture was shaken vigorously and left to stand in the dark for 30 minutes. The absorbance at 517 nm of the solution was measured in the Spectrophotometer 1500 (Unico, USA). The scavenging activity of each extract of DPPH radical was calculated using the following equation:

\[
DPPH \text{ scavenging activity} = \left( \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \right) \times 100
\]

The scavenging activity of the extracts was expressed as the EC\textsubscript{50} values, which were estimated by applying nonlinear regression (sigmoidal dose-response) to the data.

Data Analysis

All the experiments were conducted in triplicate. The statistical and nonlinear regression analyses were performed using Prism 7.0 (GraphPad Software, La Jolla, CA, USA).

RESULTS

All three alcoholic beverages were able to extract the phenolic components from the fruit and vegetable peels (Table 1). However, the ability of vodka and gin to extract the phenolic substances was either statistically comparable to or better than that of 40% laboratory grade ethanol. The tequila flavored spirit, on the other hand, was able to extract lower or statistically similar amount of phenolics compared to 40% ethanol. The labels of the three distilled spirits indicated that the ethanol content in the beverages was 40% (80 proof). The results suggest that there are other factors that determine the ability of the distilled spirits to extract phenolics from plant materials aside from their ethanol content.

Regardless of the extracting solvent, ripe mango (M. indica) peel achieved the extracts with the highest total phenolic content (34.30±0.85 to 38.65±0.48 mg GAE/g DW). The extract from squash (C. maxima) peel was found to

| Agricultural by-products | Total phenolic content (mg GAE/g DW) |
|--------------------------|-------------------------------------|
|                          | Gin | Vodka | Tequila flavored spirit | 40% Ethanol (control) |
| A. comosus               | 9.63 (0.84)*** | 10.65 (0.30)*** | 4.85 (1.02) | 6.25 (0.18) |
| C. maxima                | 3.02 (0.62)* | 2.11 (0.17) | 1.38 (0.19) | 2.15 (0.29) |
| C. microcarpa            | 18.07 (0.51)*** | 21.15(0.80)*** | 11.86(1.00)*** | 17.11 (0.47) |
| D. alata                 | 5.93 (0.61)*** | 4.79 (0.68)** | 2.06 (0.20) | 2.52 (0.56) |
| I. batatas               | 14.23 (0.54)*** | 16.63 (0.78)*** | 9.93 (0.07)*** | 14.65 (0.29) |
| M. acuminata             | 12.10(0.05)*** | 12.64 (0.61)*** | 5.85 (0.61)*** | 8.85 (0.27) |
| M. indica                | 34.30 (0.85)*** | 38.65 (0.48)*** | 35.05 (0.81) | 35.45 (0.69) |

In a row, means that are significantly different from the control (40% ethanol) based on one-way ANOVA followed by Dunnett’s Test are labeled with * (p ≤ 0.05), ** (p ≤ 0.01), and *** (p ≤ 0.001).
have the lowest phenolics content (1.38±0.19 to 3.02±0.62 mg GAE/g DW). The following sequence shows the plant materials in decreasing order of their phenolics content: \( M. \text{ indica} > C. \text{ microcarpa} > I. \text{ batatas} > M. \text{ acuminata} > A. \text{ comosus} > D. \text{ alata} > C. \text{ maxima} \).

The antioxidant property of the extracts was evaluated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay. Different concentrations of the extract were tested and the half maximal effective concentration (EC\(_{50}\)) values were estimated from the dose-response curves. The dose-response curves of the vodka extracts (Fig. 1A) are found to the right of the curves for BHA, indicating that the synthetic antioxidant exhibited stronger DPPH scavenging activity than any of the extracts. The curve for \( M. \text{ indica} \) is closest to the curve for the positive control, while the curve for \( C. \text{ microcarpa} \) is farthest. A similar trend was observed for the gin extracts (Fig. 1B). The positive control, BHA, was found to have the lowest EC\(_{50}\) value (Table 2), suggesting that it is the most effective in scavenging DPPH among the samples tested. Regardless of the solvent, \( M. \text{ indica} \) showed the lowest EC\(_{50}\) value among the plant extracts while \( C. \text{ microcarpa} \) had the highest EC\(_{50}\) value (Table 2). Taken together, these findings indicate that \( M. \text{ indica} \) has the strongest activity among the extracts, while \( C. \text{ microcarpa} \) has the weakest scavenging capacity.

The antioxidant activity of the extracts at 1000 and 100 ppm concentrations was compared to the activity of BHA at similar concentrations. At 1000 ppm concentration, the \( M. \text{ indica} \) extract exhibited DPPH scavenging activity that was statistically similar to that of BHA (Table 3). All the other extracts at 1000 ppm concentration had significantly lower activity than the positive control. At 100 ppm level, the scavenging activity of the extracts exhibited significantly weaker activity than the positive control (Table 3).

**DISCUSSION**

Because phenolics are abundant and ubiquitous in plants, their presence in the fruit and vegetable peels is not unexpected. The extraction of phenolic antioxidant compounds in the peels of mango (\( M. \text{ indica} \)) [Ajila et al., 2010; Ajila et al. 2007a; Ajila et al. 2007b; Ashoush and Gadallah, 2011; Kim et al., 2010; Ribeiro et al., 2008; Sogi et al., 2013], banana (\( M. \text{ acuminata} \))

![Figure 1](image.png)

**Figure 1.** Concentration-response curves of the fruit and vegetable peels extracted with vodka (A) and gin (B) against their DPPH scavenging activity
Table 2. Estimates of the half maximal effective concentrations (EC$_{50}$, ppm) of the DPPH scavenging activity of the different extracts

| Agricultural by-products | EC$_{50}$ (ppm) |
|--------------------------|----------------|
|                          | Vodka | Gin  |
| M. indica                | 15.67 | 17.89|
| I. batatas               | 175.5 | 745.9|
| M. acuminata             | 271.1 | 381.1|
| C. microcarpa            | 801.5 | 815.9|
| BHA (control)            | 1.66  | 2.83 |

Table 3. The DPPH scavenging activity of gin and vodka extracts from fruit and vegetable peels at 1000 and 100 ppm, compared with the activity of butylated hydroxyanisole (BHA)

| Agricultural by-products | DPPH scavenging activity (%) |
|--------------------------|-------------------------------|
|                          | 1000 ppm | 100 ppm |
|                          | Gin       | Vodka    | Gin       | Vodka    |
| M. indica                | 96.06±0.17 | 94.88±0  | 94.03±0.45* | 88.95±4.23* |
| I. batatas               | 56.85±5.24*** | 79.50±6.68*** | 12.65±1.14*** | 39.29±4.14*** |
| C. microcarpa            | 53.88±1.47*** | 52.11±3.67*** | 13.57±2.40*** | 18.50±0.10*** |
| M. acuminata             | 81.80±2.13*** | 79.53±0.37*** | 9.39±1.04*** | 26.39±0.65*** |
| BHA (control)            | 98.40±0.06 | 97.63±0.10 | 97.52±0.15 | 97.53±1.70 |

In a column, means that are significantly different from the control (BHA) based on one-way ANOVA followed by Dunnett’s Test are labeled with * (p ≤ 0.05), ** (p ≤ 0.01), and *** (p ≤ 0.001).
activity was exhibited by calamansi (C. microcarpa) peel. The results of the DPPH activity assay agree with a previous report that among mango (M. indica), banana (M. acuminata) and calamansi (C. microcarpa) peels, mango had the strongest activity while calamansi had the weakest [Samonte and Trinidad, 2013]. The mango (M. indica) peel extract was found to exert statistically comparable antioxidant activity with BHA at 1000 ppm level, but not at 100 ppm. This is expected since crude extract was used. Since other substances in the crude extract do not possess the antioxidant property, a large amount of the crude extract is needed for its activity to become comparable with the positive control.

CONCLUSION

Agricultural residues are rich in phenolics with antioxidant properties. Although alcoholic beverages have traditionally been used in herbal medicine preparation, their use in the extraction of phenolic compounds has not received much attention. In this study, the ability of gin, vodka, and tequila flavored spirits, which contain 40% alcohol, to extract phenolics from the selected fruit and vegetable wastes was evaluated. Although the three beverages were able to extract the phenolic substances from plant materials, gin and vodka had comparable or better extraction power compared with 40% reagent grade ethanol. Regardless of the solvent, the mango peel enabled to obtain the extracts with the highest phenolics content and strongest antioxidant activity. The findings may serve as basis for the development of technology for the utilization of natural antioxidants from waste materials using edible solvents.

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